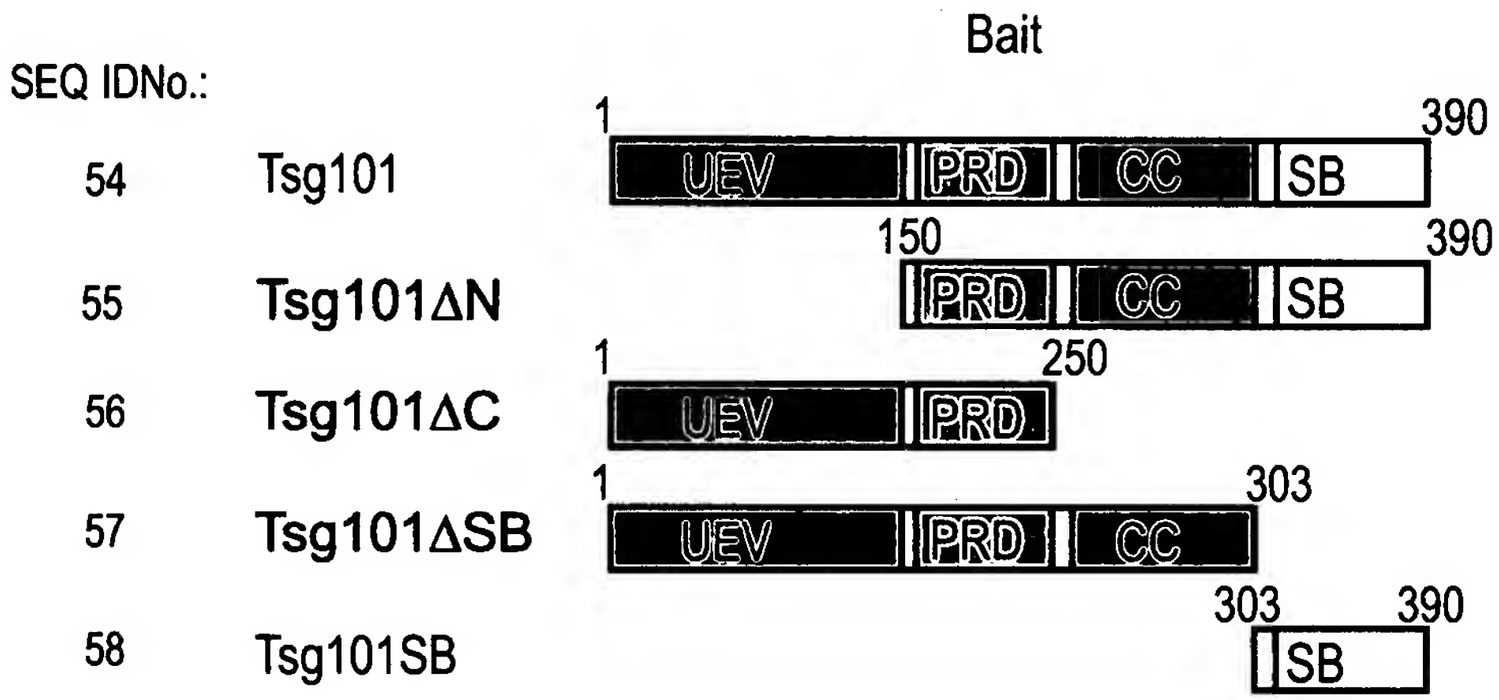


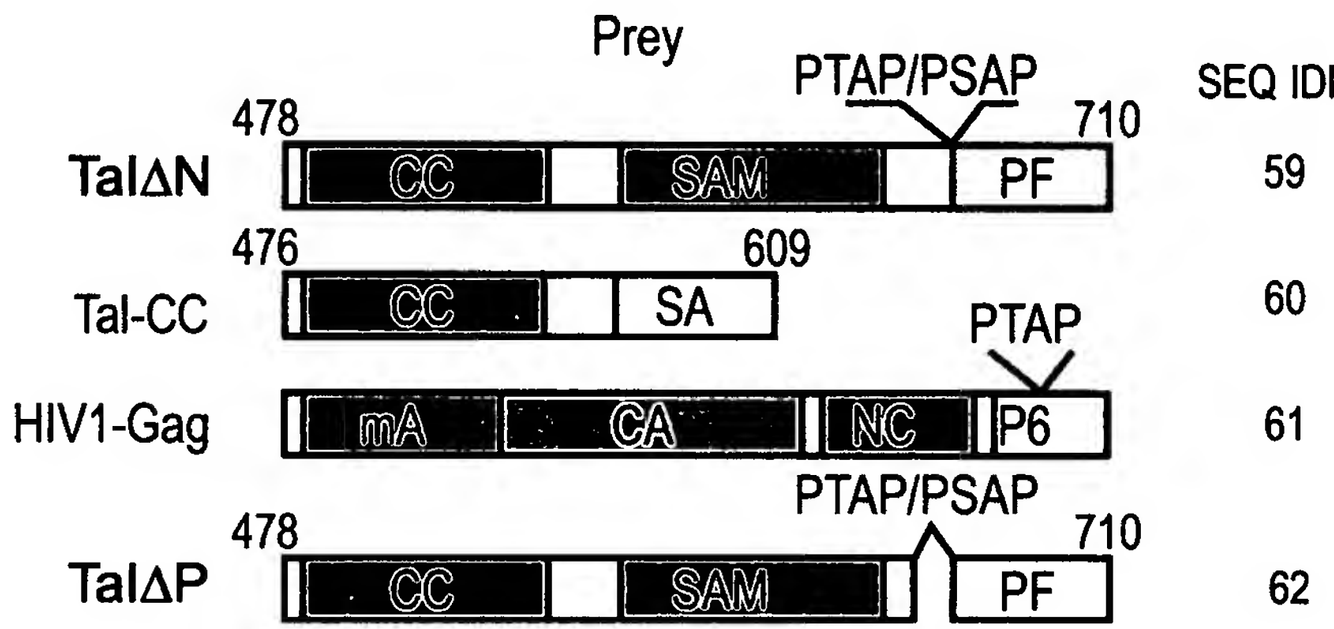


Fig. 1a

Serial No.: 10/568,707
Inventor: YARDEN Yosef et al
Title: POLYNUCLEOTIDES, POLYPEPTIDES AND ANTIBODIES AND USE THEREOF IN TREATING TSG101-ASSOCIATED DISEASES
REPLACEMENT DRAWING
Sheet: 1 of 25
Attorney Docket No. 31570

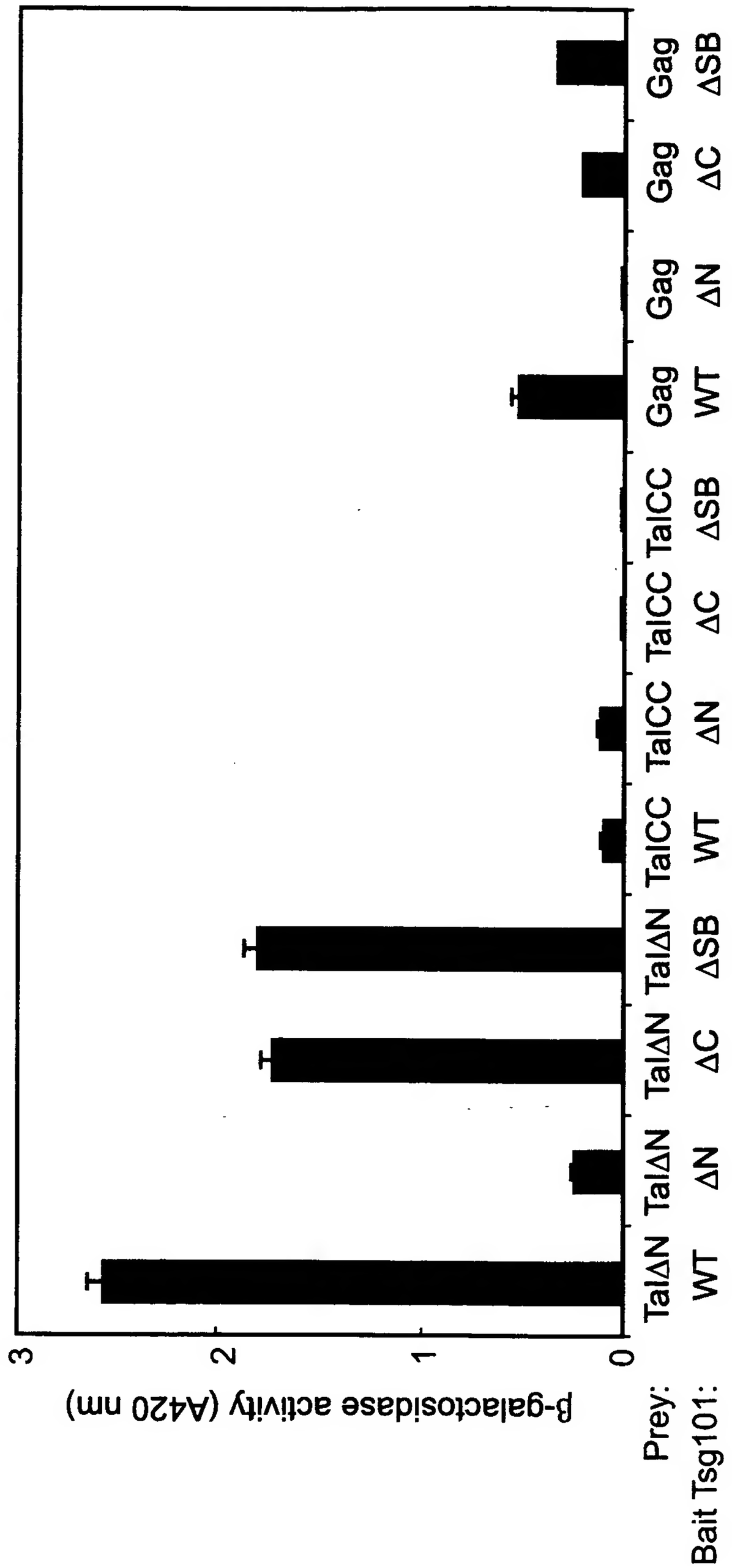

β-galactosidase activity (A420 nm)
0
1
2
3
Bait Tsg101:
WT
TalΔN
ΔN
TalΔN
ΔC
TalΔN
ΔSB
TalΔN
Prey:
WT
TalCC
ΔN
TalCC
ΔC
TalCC
ΔSB
TalCC
Gag
WT
Gag
ΔSB
Gag
ΔC
Gag
ΔN

Fig. 1b

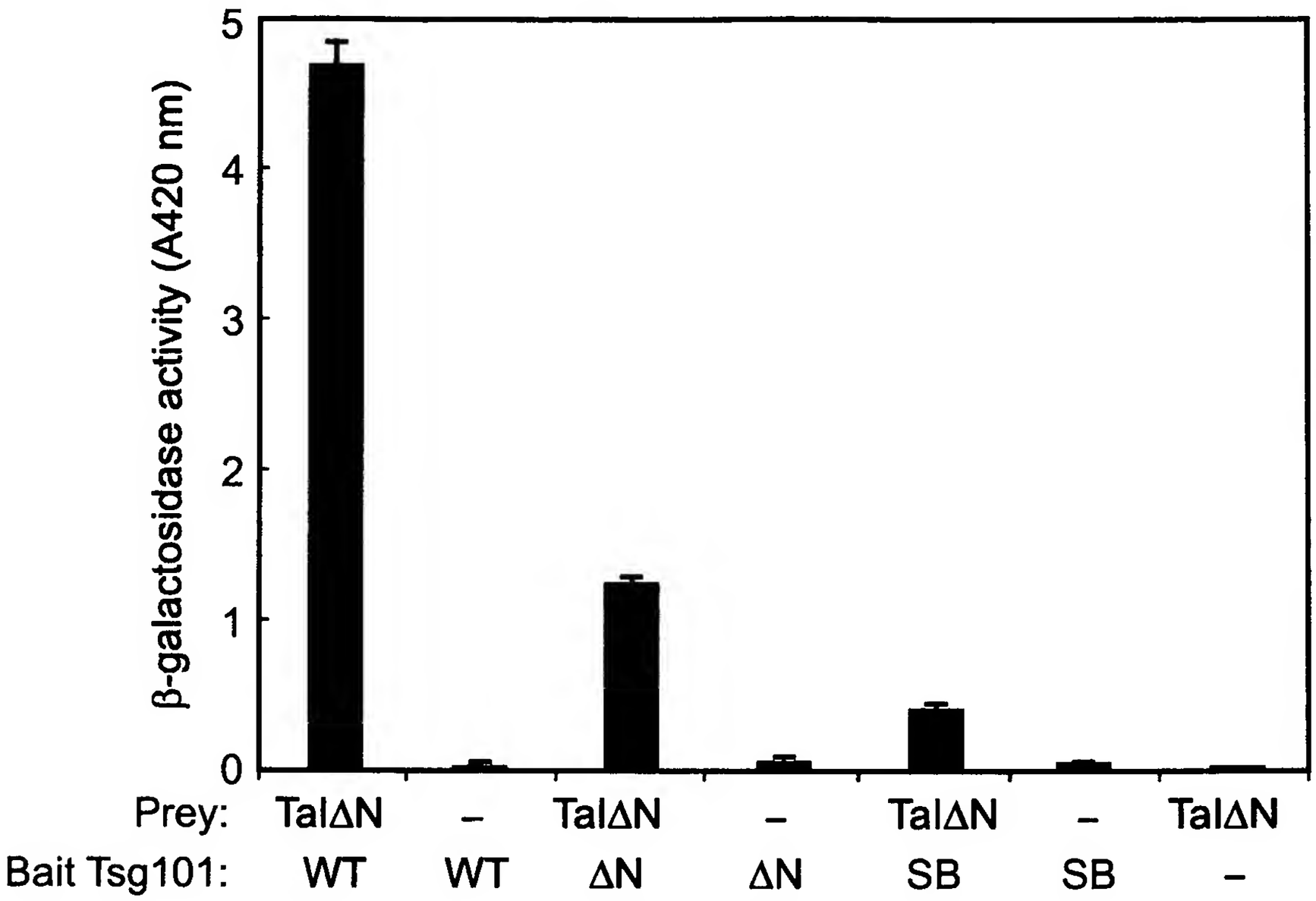

β-galactosidase activity (A420 nm)
5
4
3
2
1
0
Prey: TalΔN – TalΔN – TalΔN – TalΔN
Bait Tsg101: WT WT ΔN ΔN SB SB –

Fig. 1c

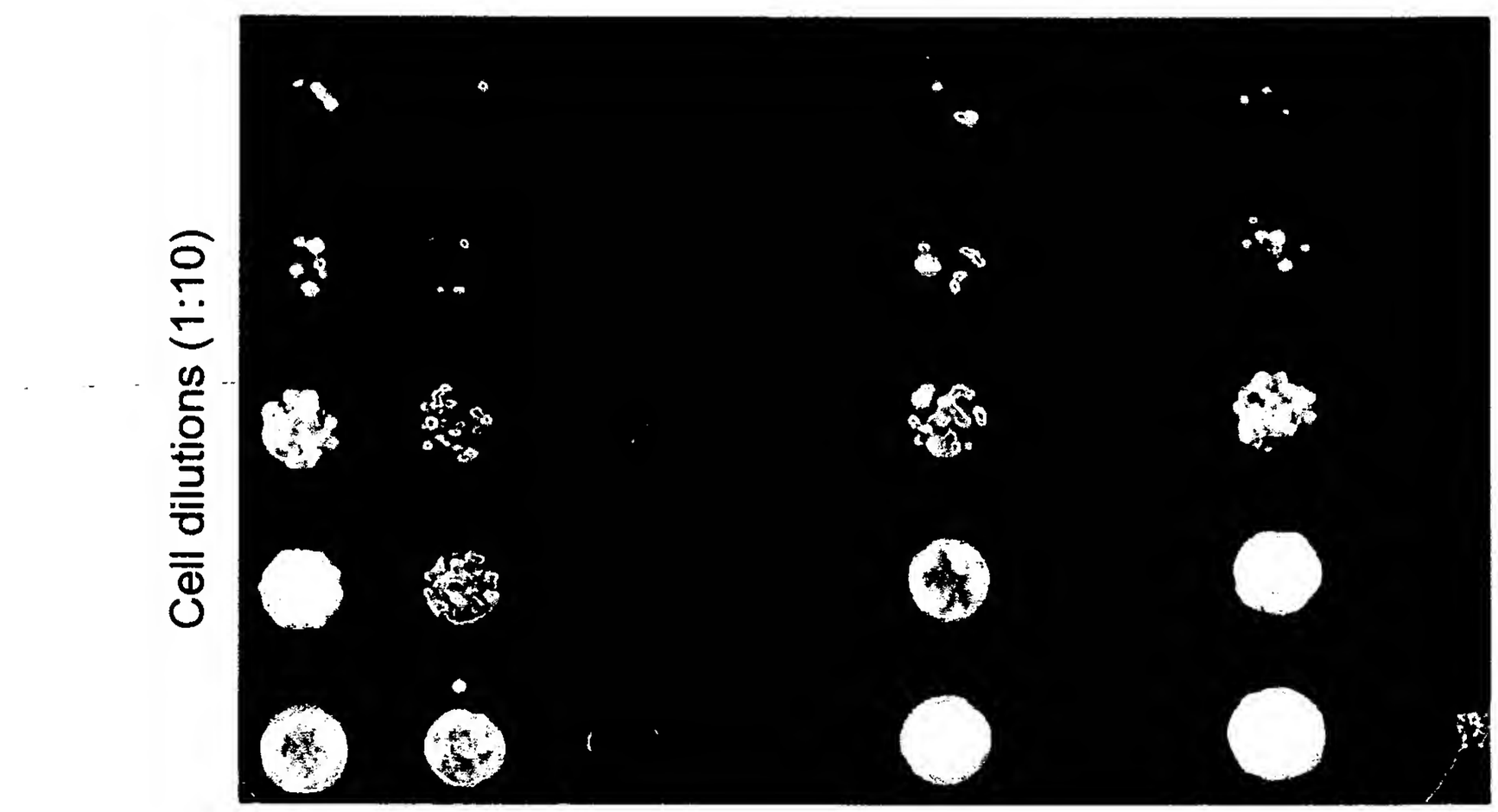

Cell dilutions (1:10)
Prey: ΔN ΔP ΔN ΔP ΔN ΔP ΔN ΔP
Bait Tsg101: WT WT ΔN ΔN ΔSB ΔSB ΔC ΔC

Fig. 1d

```
   1  MPLFFRKRKP SEEARKRLEY QMCLAKEAGA DDILDISKCE LSEIPFGAFA
  51  TCKVLQKKVL IVHTNHLTSL LPKSCSLLSL ATIKVLDLHD NQLTALPDDL
 101  GQLTALQVLN VERNQLMQLP RSIGNLTQLQ TLNVKDNKLK ELPDTVGELR
 151  SLRTLNISGN EIQRLPQMLA HVRTLEMLSL DASAMVYPPR EVCGAGTAAI
 201  LQFLCKESGL EYYPPSQYLL PILEQDGIEN SRDSPDGPTD RFSREELEWQ
 251  NRFSDYEKRK EQKMLEKLEF ERRLELGQRE HTQLLQQSSS QKDEILQTVK
 301  EEQSRLEQGL SEHQRHLDAE RQRLQEQLKQ TEQNISSRIQ KLLQDNQRQK
 351  KSSEILKSLE NERIRMEQLM SITQEETESL RRRDVASAMQ QMLTESCKNR
 401  LIQMAYESQR QNLVQQACSS MAEMDERFQQ ILSWQQMDQN KAISQILQES
 451  AMQKAAFEAL QVKKDLMHRQ IRSQIKLIET ELLQLTQLEL KRKSLDTESL
 501  QEMISEQRWA LSSLLQQLLK EKQQREEELR EILTELEAKS ETRQENYWLI
 551  QYQRLLNQKP LSLKLQEEGM ERQLVALLEE LSAEHYLPIF AHHRLSLDLL
 601  SQMSPGDLAK VGVSEAGLQH EILRRVQELL DAARIQPELK PPMGEVVTPT
 651  AFQEPPESVR PSAPPAELEV QASECVVCLE REAQMIFLNC GHVCCCQQCC
 701  QPLRTCPLCR QDIAQRLRIY HSS
```

SEQ ID No.: 2

Fig. 2a

SEQ ID No.: 2

Fig. 2b

```
SEQ IDNo.:4   Mouse  MPL.FFRKRK PSEEARKRLE YQMCLAKEAG ADDILDISKC ELSEIPFGAF ATCKVLQKKV LIVHTNHLTS LLPKSCSLLS  79
SEQ IDNo.:6   Rat    MPL.FFRKRK PSEEARKRLE YQMCLAKEAG ADDILDISKC ELSEIPFGAF ATCKVLQKKV LIVHTNHLTS LLPKSCSLLS  79
SEQ IDNo.:2   Human  MPL.FFRKRK PSEEARKRLE YQMCLAKEAG ADDILDISKC ELSEIPFGAF ATCKVLQKKV LIVHTNHLTS LLPKSCSLLS  79
SEQ IDNo.:63  Cinte  MPLGRFGSKK QSKNAKRRFE HLMVMAKE.N PDRIFDLSDC ELHEVPNLMF SQCRVLLTES LLLHSNLLKS .LKHGGKMSC  78

              Mouse  LVTIKVLDLH ENQLTALPDD MGQLTVLQVL NVERNQLTHL PRSIGNLLQL QTLNVKDNKL KELPDTLGEL RSLRTLDISE 159
              Rat    LATIKVLDLH DNQLTALPDD IGQLTALQVL NVERNQLTHL PRSVGNLLQL QTLNVKDNKL KELPDTLGEL RSLRTLDISE 159
              Human  LATIKVLDLH DNQLTALPDD LGQLTALQVL NVERNQLMQL PRSIGNLTQL QTLNVKDNKL KELPDTVGEL RSLRTLNISG 159
              Cinte  LTSLRVLDLH NNRIALLPKD LGVLSNLQVF NIENNRITEL PDSLGDLKKL QSLLAKDNQL NSLPTTISGM ESLRTLDLSG 158

              Mouse  .NEIQRLPQM LAHVRTLEVL SL.NALAMVY PPPEVCGAGT AAVQQFLCKE SGLDYYPPSQ YLLPVLEQDG AENTQDSPDG 237
              Rat    .NEIQRLPQM LAHVRTLEVL SL.DALSMVY PPPEVCGAGT AAVQQFLCKE SGLDYYPPSQ YLLPVLEQDG AENSQDSPDG 237
              Human  .NEIQRLPQM LAHVRTLEML SL.DASAMVY PPREVCGAGT AAILQFLCKE SGLEYYPPSQ YLLPILEQDG IENSRDSPDG 237
              Cinte  TNKVLYLPKT LCKVRTLEVF VLSNPAVMEY PHSMVACEGL EAIQKFICKD TGIEYIPPSH ATLKVLDSSA TTSSSSKQTA 238

              Mouse  PASRFSREEA EWQNRFSDYE KRKEQKMLEK LEFERRLDLG QREHAELLQQ SHSHKDEILQ TVKQEQTRLE QDLSERQRCL 317
              Rat    PTRRFSREEA EWQNRFSDYE KRKEQKMLEK LEFERRLDLG QREHAELLQQ SHSHKDEILQ TVKQEQTRLE QGLSERQRCL 317
              Human  PTDRFSREEL EWQNRFSDYE KRKEQKMLEK LEFERRLELG QREHTQLLQQ SSSQKDEILQ TVKEEQSRLE QGLSEHQRHL 317
              Cinte  AANL_____Q LYQSSMDQYQ RSKDEKMKQQ LETERSIAEQ QREQAYLTAK AKHEKAKENA FLKQEQAVFD RSISEQQKKT 313

              Mouse  DAERQQLQEQ LKQTEQSIAS RIQRLLQDNQ RQKKSSEILK SLENERIRNE QLMSITQEET ENLRQREIAA AMQQMLTESC 397
              Rat    DAERQQLQEQ LKQSEQSIAS RIQRLLQDNQ RQKKSSEILK SLENERIRNE QLMSITQEET ENLRQREIAA AMQQMLTESC 397
              Human  DAERQRLQEQ LKQTEQNLSS RIQKLLQDNQ RQKKSSEILK SLENERIRNE QLMSITQEET ESLRRRDVAS AMQQMLTESC 397
              Cinte  EIERQEMMKT LTQVEEEASR LVNKLVSMNI GAKQREEMLE GMERERMEQE ERFKVTQEDI DKLRKKETLA AMQSVLADNA 393

              Mouse  KSRLIQMAYE SQRQSLAQQA CSSMAEMDKR FQQILSWQQM DQNKAISQIL QESVMQKAAF EALQVKKDLM HRQIRNQIRL 477
              Rat    KSRLIQMAYE SQRQSLVQQA CSSMAEMDKR FQQILSWQQM DQNKAISQIL QESVMQKAAF EALQVKKDLT HRQIRSQIRL 477
              Human  KNRLIQMAYE SQRQNLVQQA CSSMAEMDER FQQILSWQQM DQNKAISQIL QESAMQKAAF EALQVKKDLM HRQIRSQIKL 477
              Cinte  HYAIAIKKYL GEQDHMTRQA QQTLGADNEL IEHELKRQQW NQGVLVDQIL HEESLQKEAF IMLKLQHDAV QARLVDQIGQ 473

              Mouse  IETELLQLTQ LELKRKSLDT ETLQEMVSEQ RWALSNLLQQ LLKEKKQREE ELHGILAELE AKSETKQENY WLIQYQRLLN 557
              Rat    IETELLQLTQ LELKRKSLDT ETLQEMVSEQ RWALSNLLQQ LLKEKKQREE ELHGILAELE AKSETKQENY WLIQYQRLLN 557
              Human  IETELLQLTQ LELKRKSLDT ESLQEMISEQ RWALSSLLQQ LLKEKQQREE ELREILTELE AKSETRQENY WLIQYQRLLN 557
              Cinte  LQGELIRLTQ IEAQRNKHRI DQDKQTLSLI RNELTDLLIQ LLKEKDHREE MVKSRLVEME QQREDDQVDF WLVQYQKLLD 553

              Mouse  QKPLSLKLQE EGMERRLVAL LVELSAEHYL PLFAHHRISL DMLSRMSPGD LAKVGVSEAG LQHEILRRAQ DLLAVPRVQP 637
              Rat    QKPLSLKLQE EGMERQLVAL LVELSAEHYL PLFAHHRITL DMLSRMGPGD LAKVGVSEAG LQHEILRRAR DLLDVARVQP 637
              Human  QKPLSLKLQE EGMERQLVAL LEELSAEHYL PIFAHHRLSL DLLSQMSPGD LAKVGVSEAG LQHEILRRVQ ELLDAARIQP 637
              Cinte  TKPEVLVQKE HGVDPQIVRL LQRSDAAHHL SAEARHHITM DTIITTLDDEK LRSLGVFEIG LRRNLLREIE ELY.ILQRKKV 632

              Mouse  ELKPLENEVL GALEPPTAPR EL.....QES VRPSAPPAEL DMPTSECVVC LEREAQMVFL TCGHVCCCQQ CCQPLRTCPL 712
              Rat    ELKPPKNEVF GVSEPPTAPQ EL.....PES VRPSAPPAEL DVPTSECVVC LEREAQMVFL TCGHVCCCQQ CCQPLRTCPL 712
              Human  ELKPPMGEVV ....TPTAPQ EP.....PES VRPSAPPAEL EVQASECVVC LEREAQMIFL NCGHVCCCQQ CCQPLRTCPL 708
              Cinte  DLPTSDEE.. ..HPPPTAPV EQSTSQDPDV VQPTAP.SES QEEENECVVC LDRNSDTIFL PCGHVCACFI CSTQLQSCPM 707

              Mouse  CRQEISQRLR IYHSS 727
              Rat    CRQEISQRLR IYHSS 727
              Human  CRQDIAQRLR IYHSS 723
              Cinte  CRSDVAQKIK IFRS- 721
```

FIG. 2C

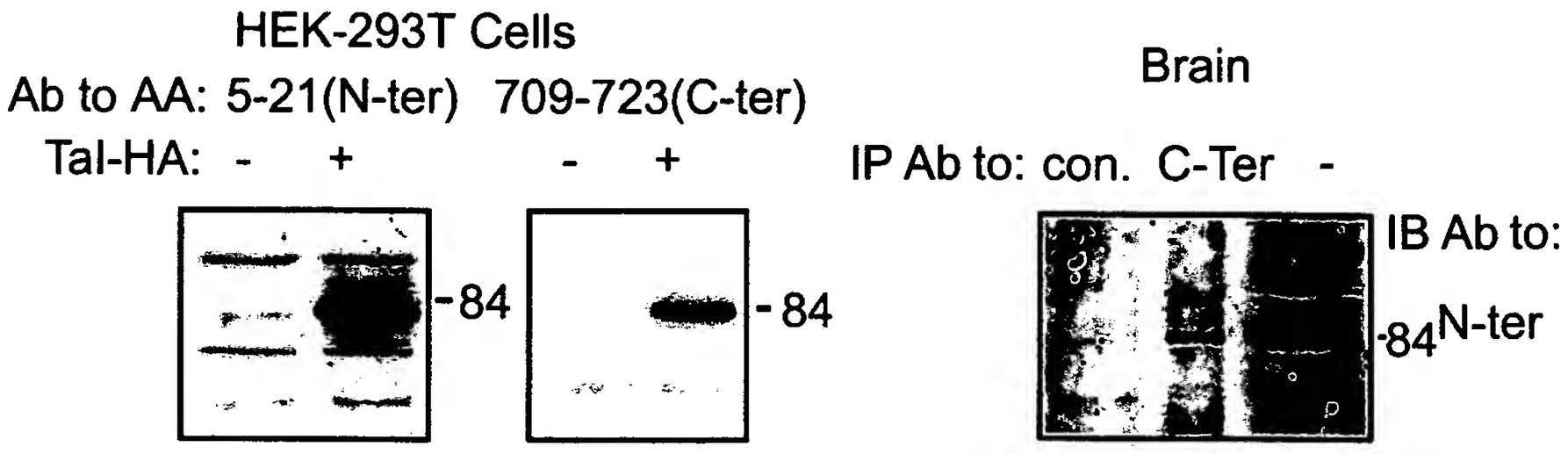


Fig. 2d

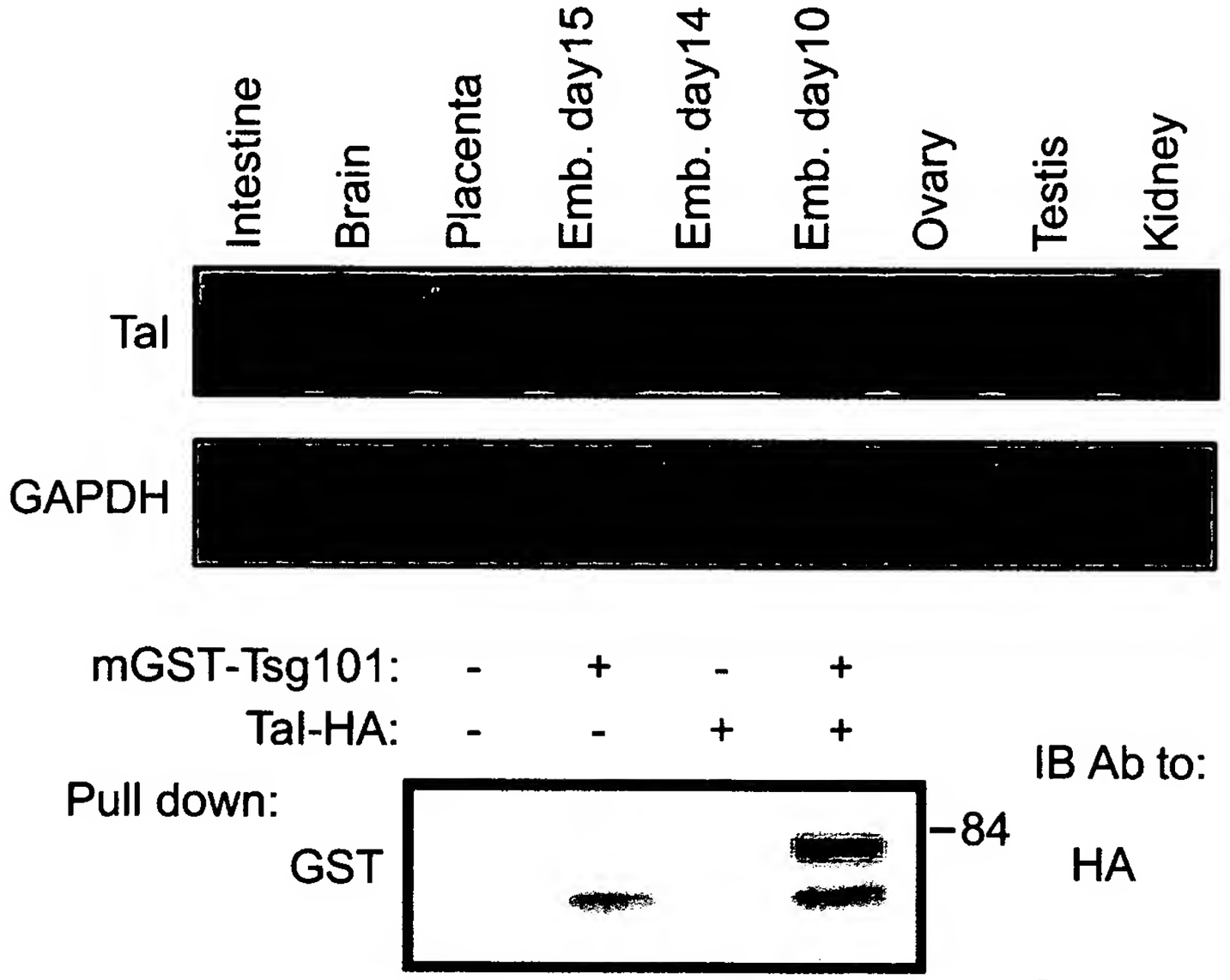


Fig. 2e

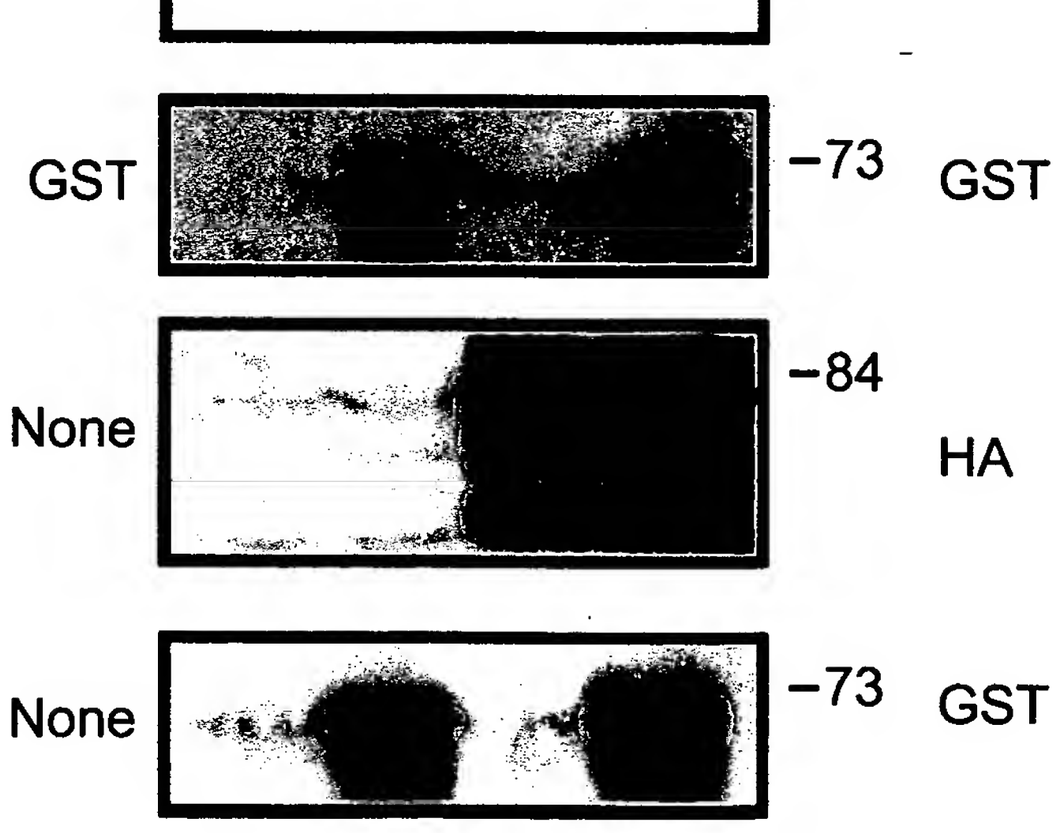


Fig. 3a

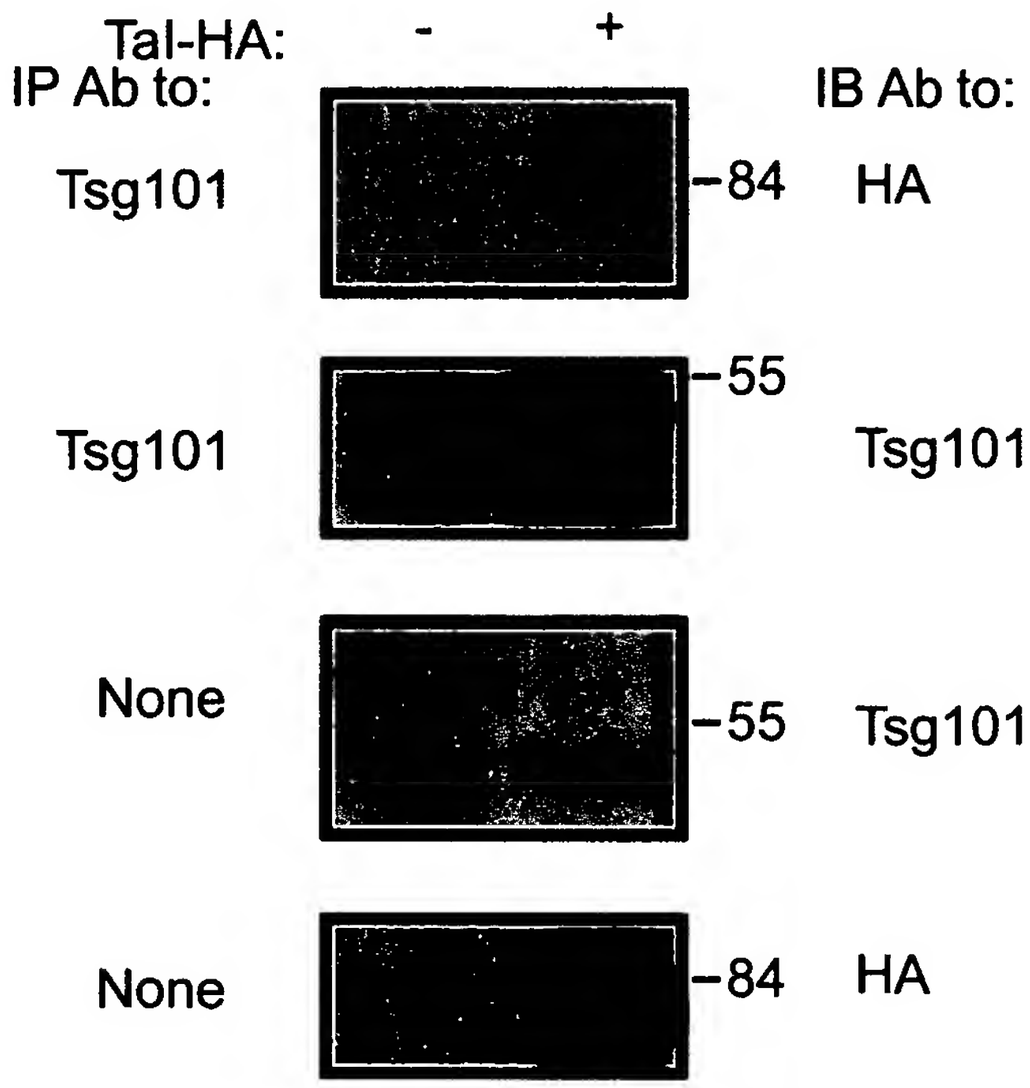

Tal-HA: - +
IP Ab to:
IB Ab to:
Tsg101
84
HA
55
Tsg101
Tsg101
None
55
Tsg101
None
84
HA

Fig. 3b

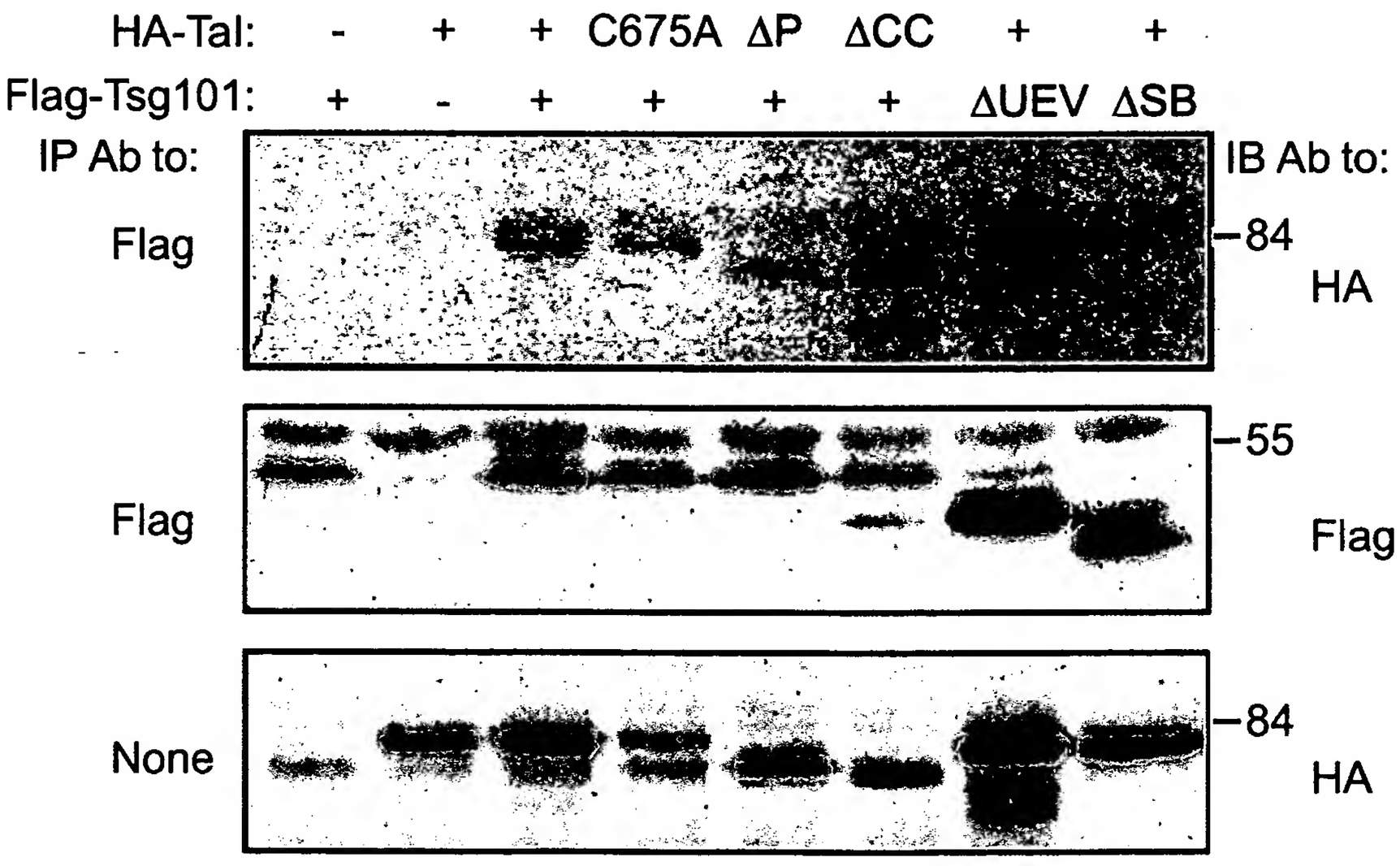

HA-Tal: - + + C675A ΔP ΔCC + +
Flag-Tsg101: + - + + + + ΔUEV ΔSB
IP Ab to:
IB Ab to:
Flag
84
HA
55
Flag
Flag
None
84
HA

Fig. 3c

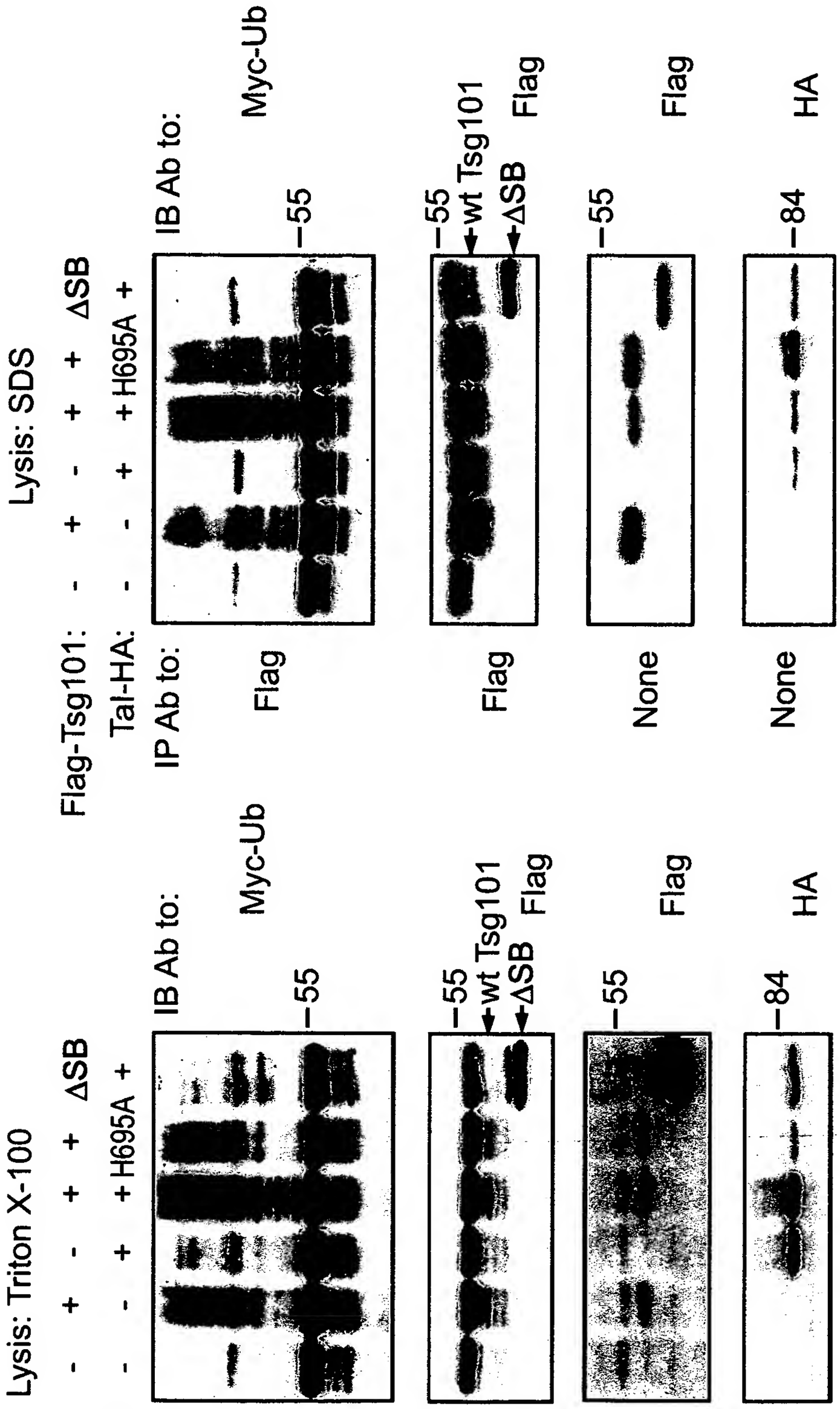

Lysis: SDS
Flag-Tsg101: - + - + + ΔSB
Tal-HA: - - + + H695A +
IP Ab to:
IB Ab to:
Flag
Myc-Ub
Flag
wt Tsg101
ΔSB
Flag
None
HA
55
84

Fig. 4b

Lysis: Triton X-100
Flag-Tsg101: - + - + + ΔSB
Tal-HA: - - + + H695A +
IP Ab to:
IB Ab to:
Flag
Myc-Ub
Flag
wt Tsg101
ΔSB
Flag
None
HA
Hsp70
None
55
84

Fig. 4a

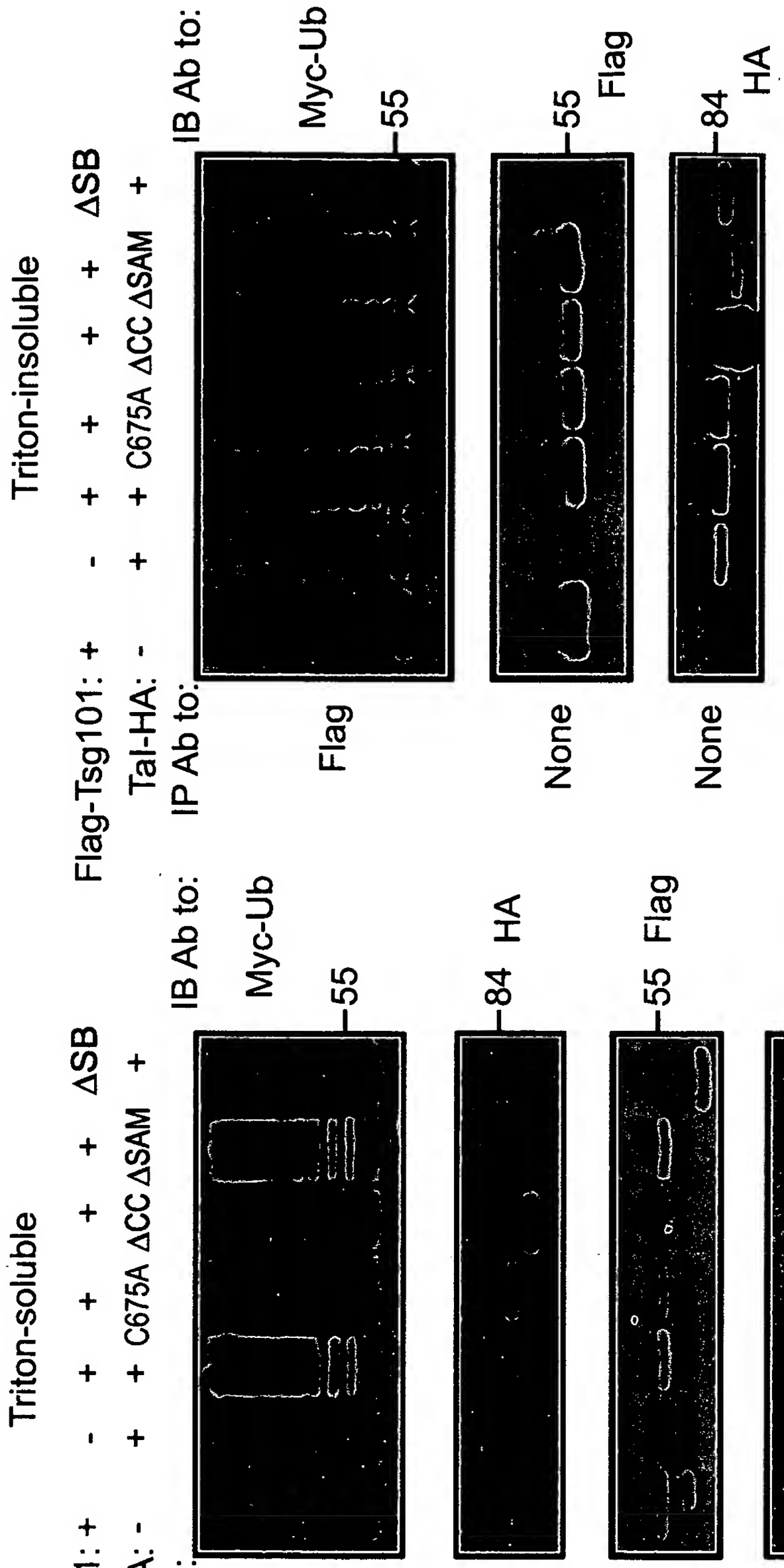

Triton-insoluble
- + + + + +
+ + C675A ΔCC ΔSAM + ΔSB +
Flag-Tsg101: + + + + + +
Tal-HA: - + + + + +
IP Ab to: Flag Flag None None
IB Ab to: Myc-Ub 84 HA 55 Flag 84 HA
55
55
84

Fig. 4c

Triton-insoluble
- + + + + +
+ + C675A ΔCC ΔSAM + ΔSB +
Flag-Tsg101: + + + + + +
Tal-HA: - + + + + +
IP Ab to: Flag Flag None None
IB Ab to: Myc-Ub 84 HA 55 Flag 84 HA
55
55
84

Fig. 4d

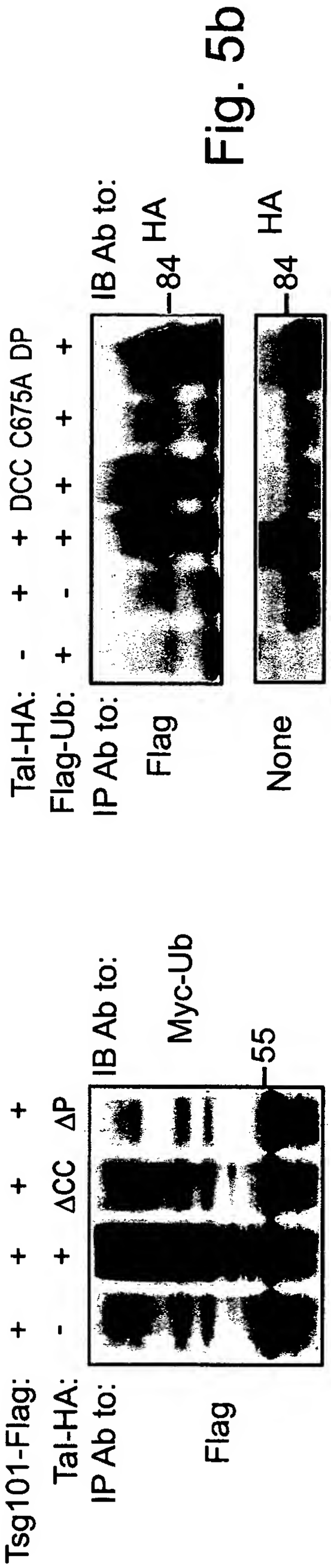


Fig. 5a

Fig. 5b

Fig. 5c

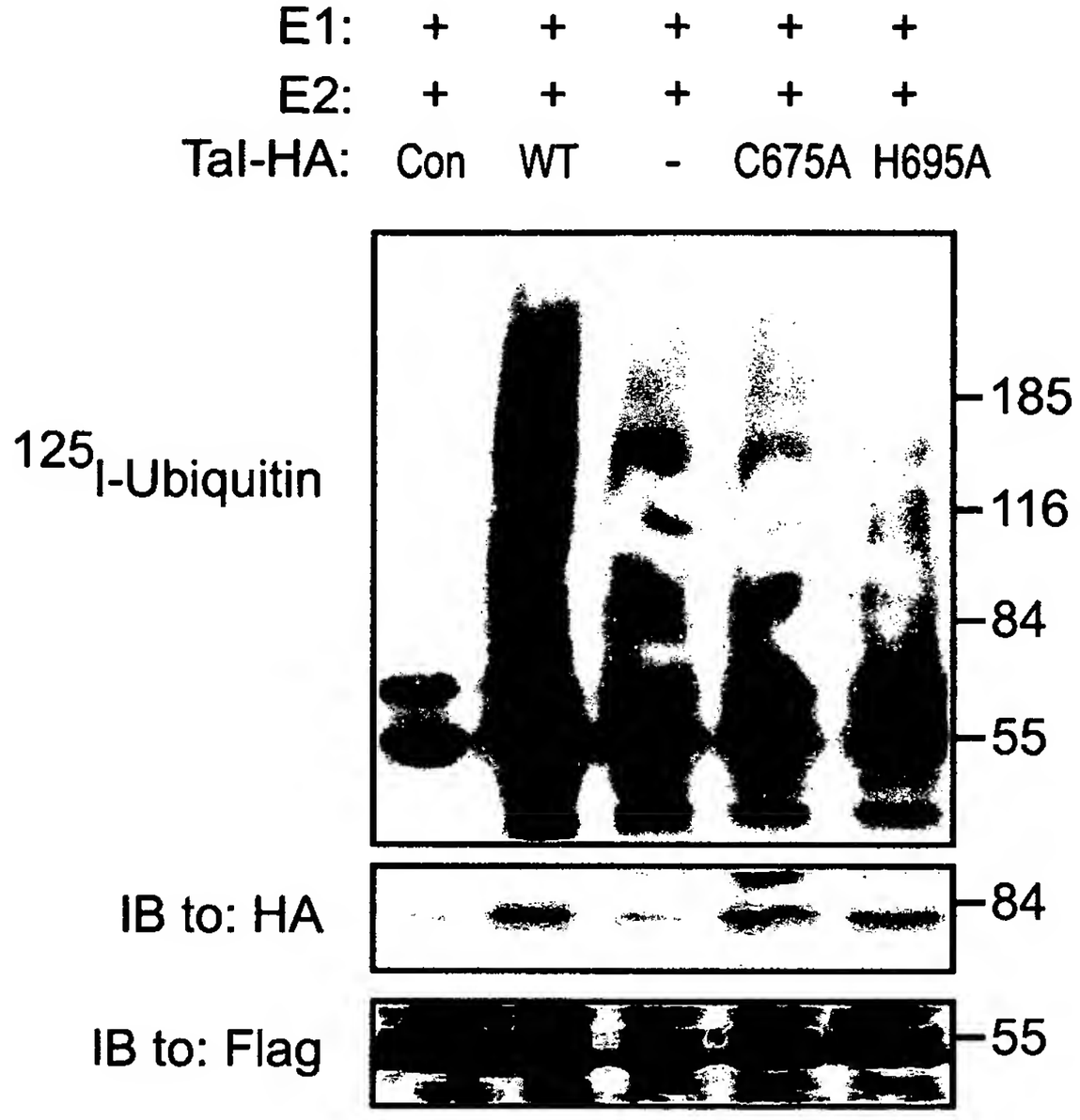

E1: + + + + +
E2: + + + + +
Tal-HA: Con WT - C675A H695A
125I-Ubiquitin
185
116
84
55
IB to: HA
84
IB to: Flag
55

Fig. 5d

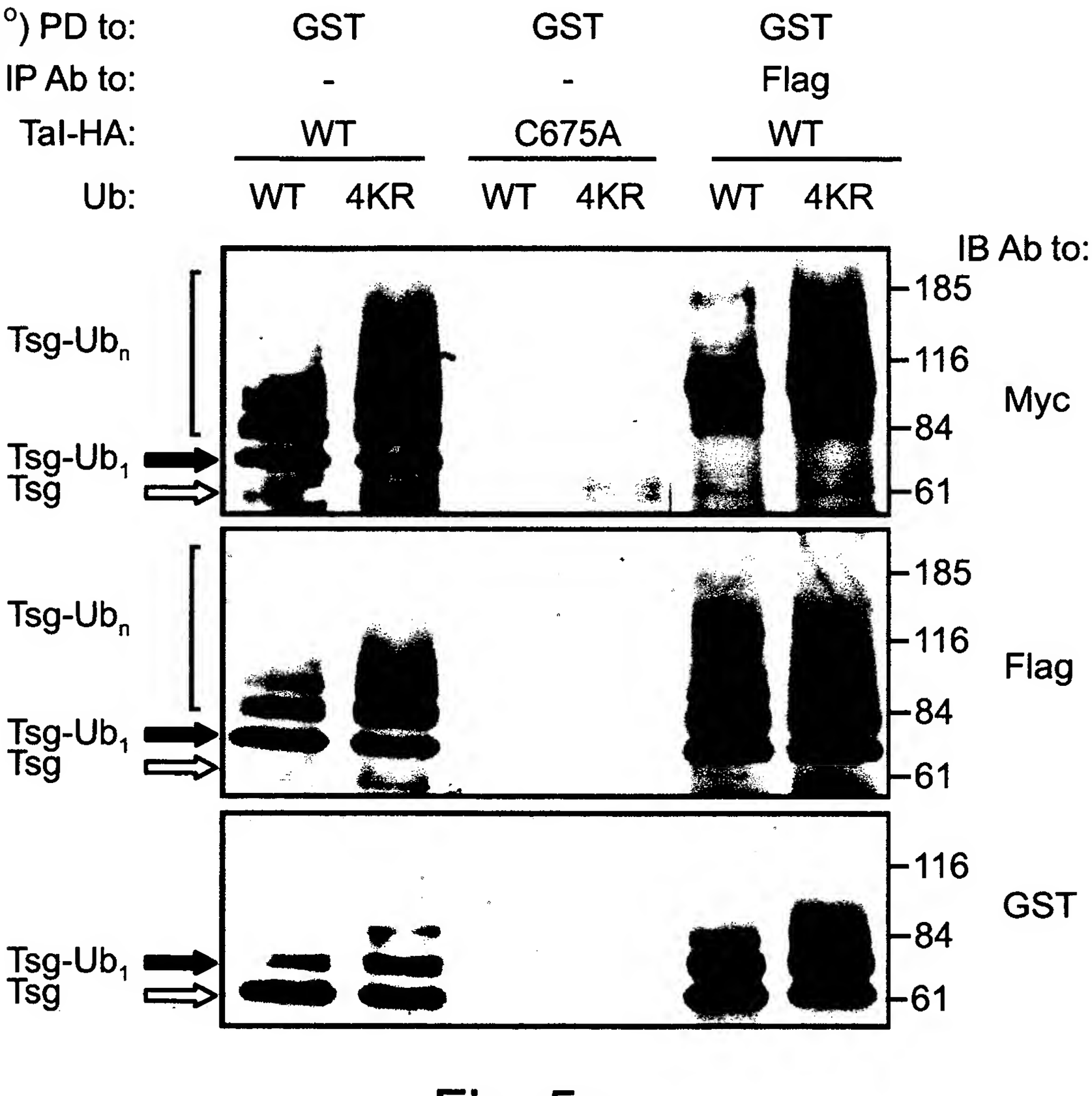

(1°) PD to:
GST
GST
GST
(2°) IP Ab to:
-
-
Flag
Tal-HA:
WT
C675A
WT
Ub:
WT 4KR
WT 4KR
WT 4KR
IB Ab to:
Tsg-Ub$_n$
Tsg-Ub$_1$
Tsg
185
116
84
61
Myc
Tsg-Ub$_n$
Tsg-Ub$_1$
Tsg
185
116
84
61
Flag
Tsg-Ub$_1$
Tsg
116
84
61
GST

Fig. 5e

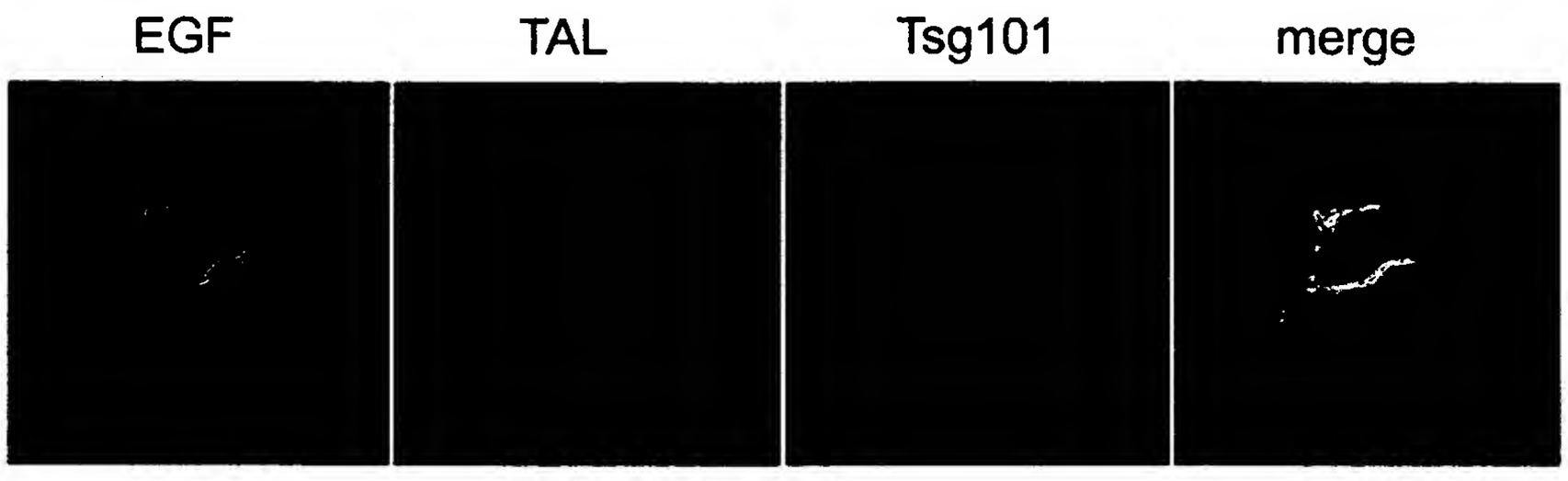


Fig. 6a

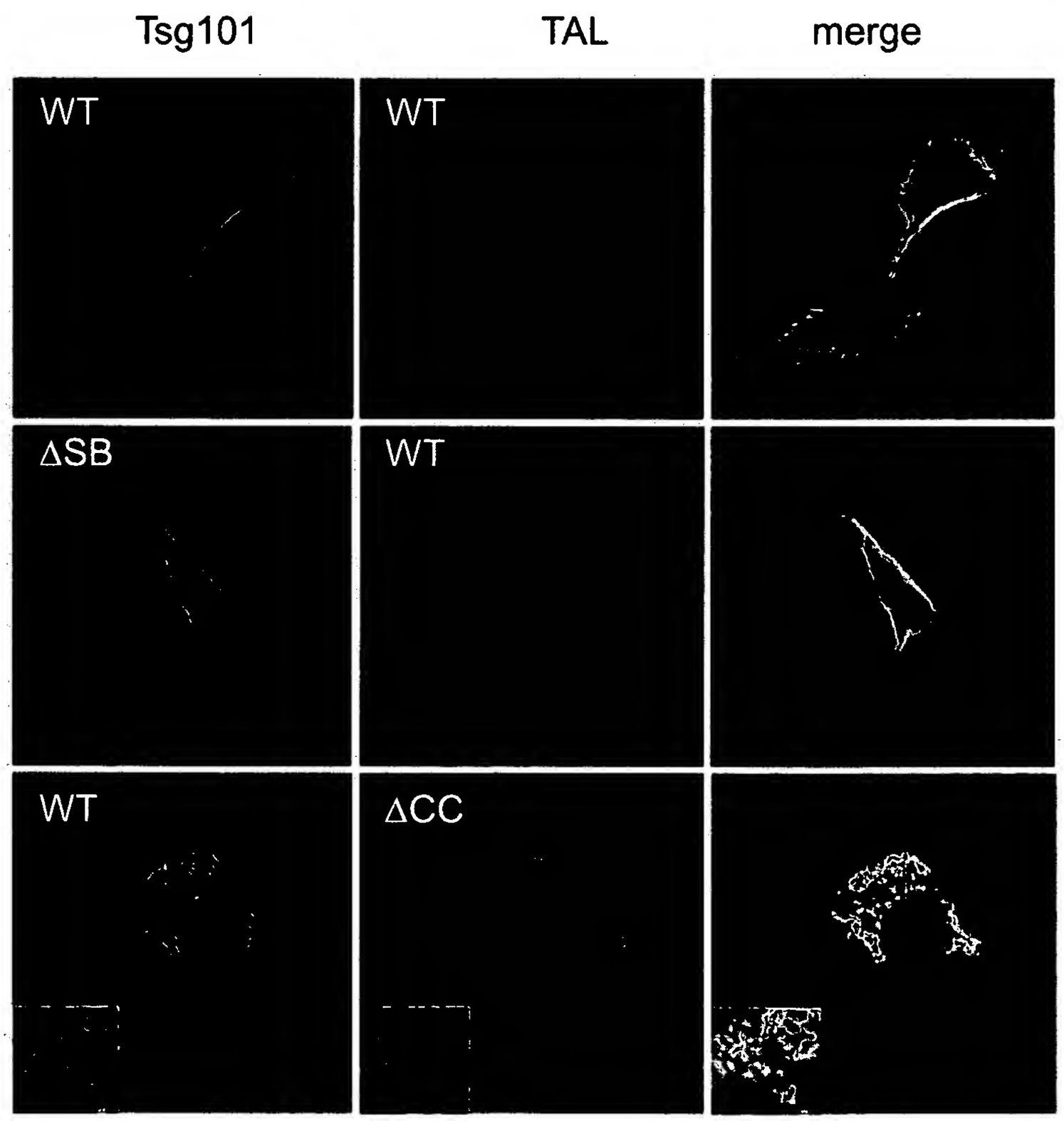


Fig. 6b

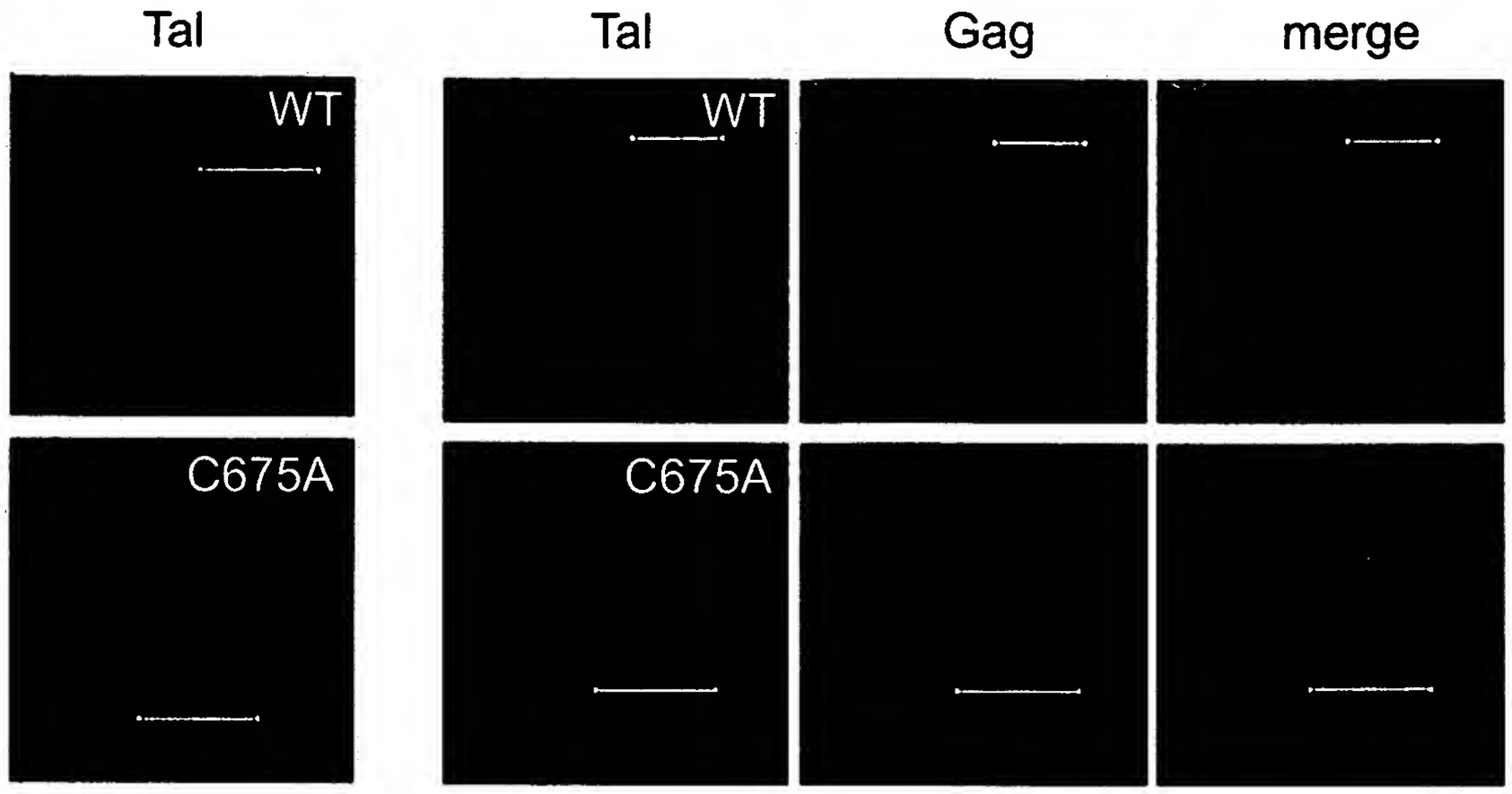


Fig. 6c

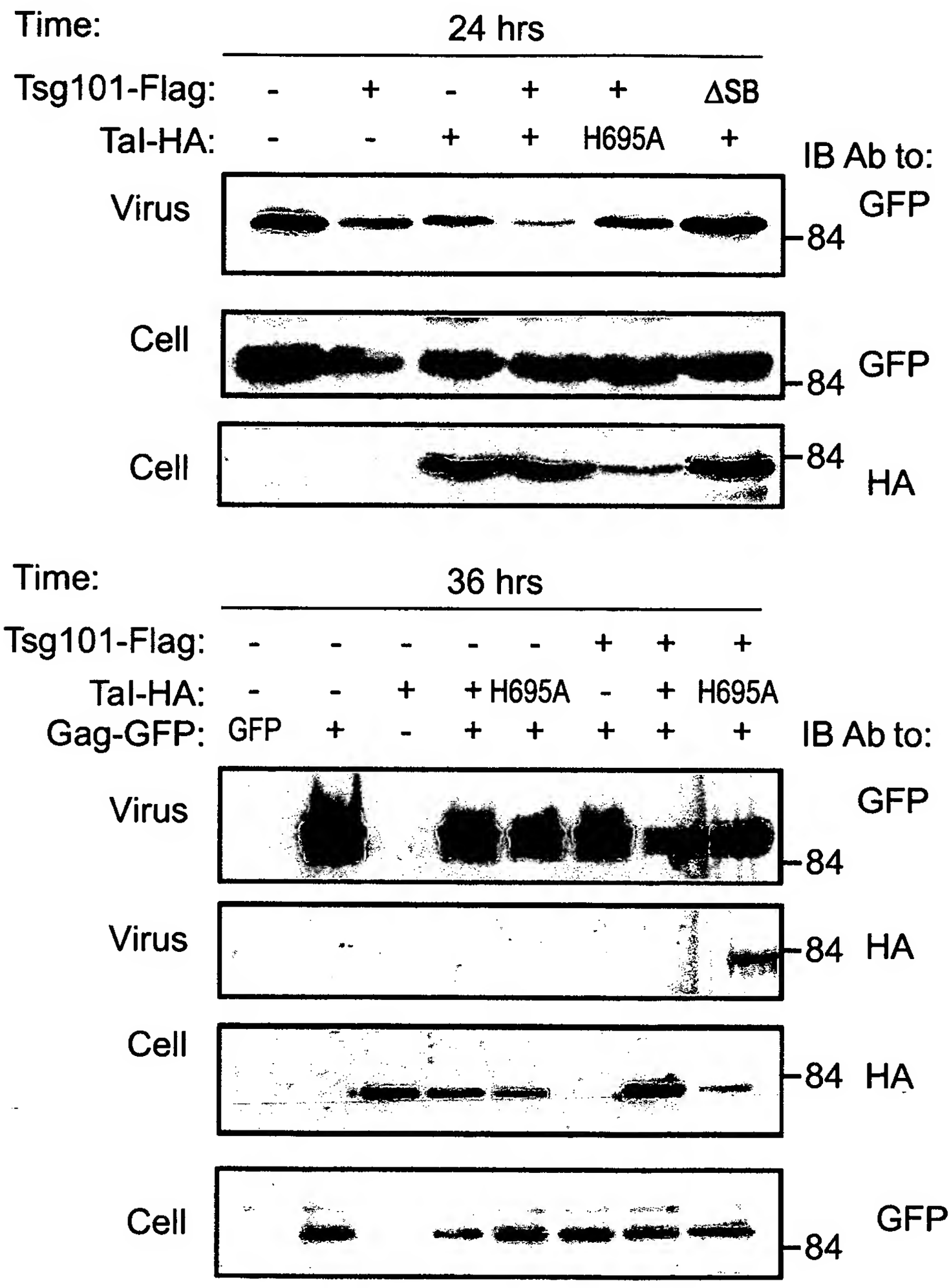

Time:
24 hrs
Tsg101-Flag: - + - + + ΔSB
Tal-HA: - - + + H695A +
IB Ab to:
Virus
GFP
84
Cell
GFP
84
Cell
84
HA
Time:
36 hrs
Tsg101-Flag: - - - - - + + +
Tal-HA: - - + + H695A - + H695A
Gag-GFP: GFP + - + + + + +
IB Ab to:
Virus
GFP
84
Virus
84
HA
Cell
84
HA
Cell
GFP
84

Fig. 7a

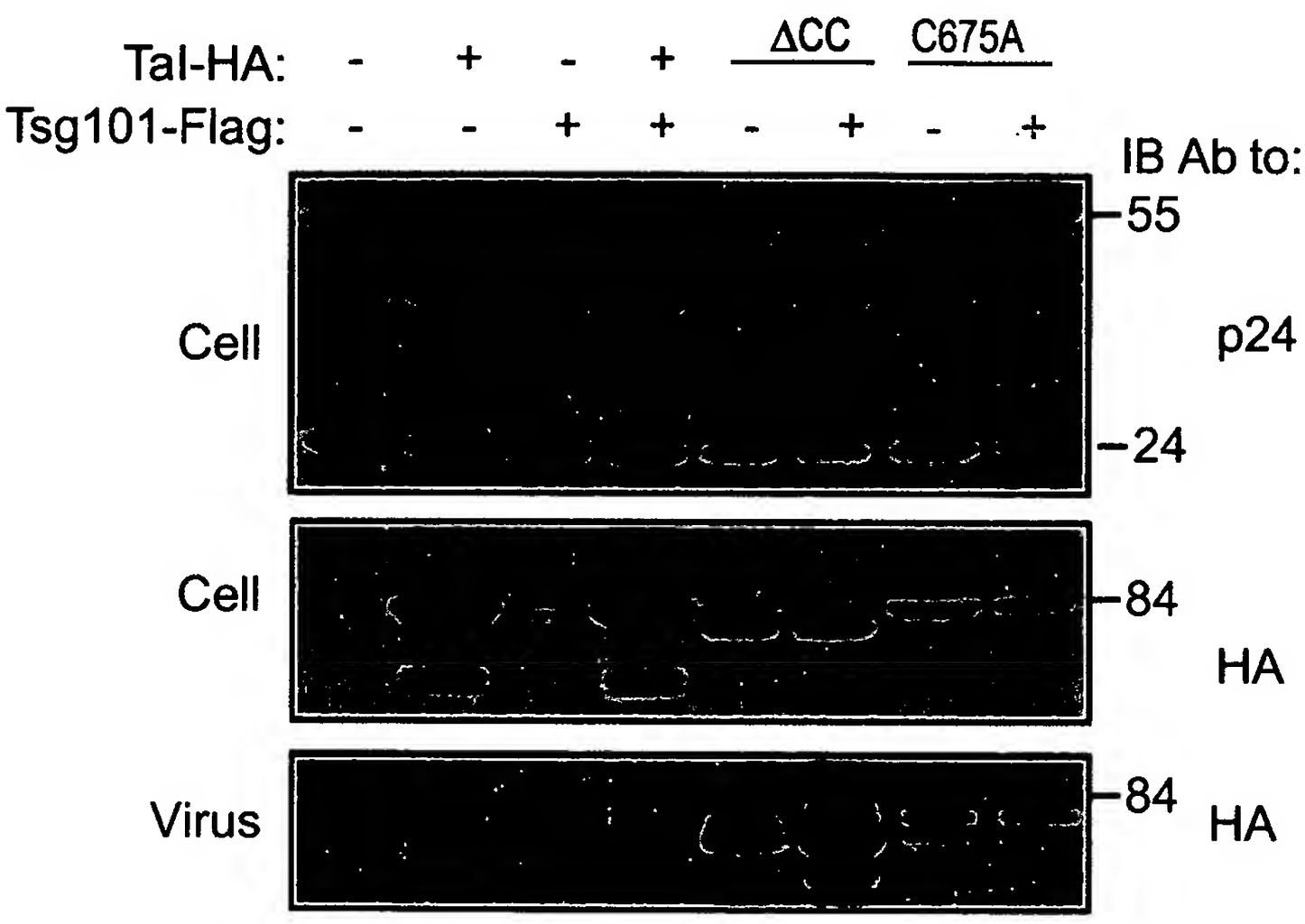

Tal-HA: - + - + ΔCC C675A
Tsg101-Flag: - - + + - + - +
IB Ab to:
Cell
55
p24
24
Cell
84
HA
Virus
84
HA

Fig. 7b

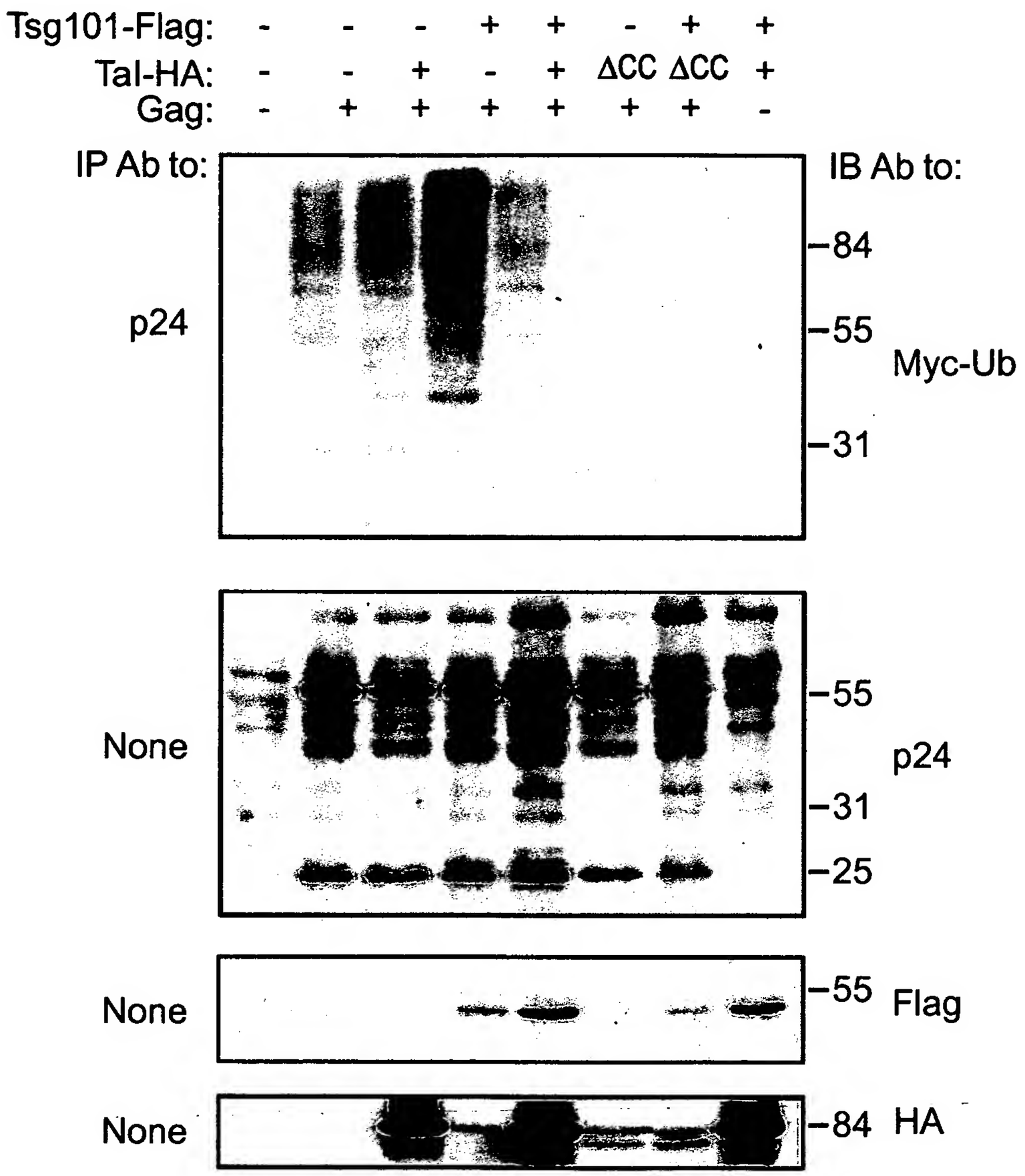

Tsg101-Flag: - - - + + - + +
Tal-HA: - - + - + ΔCC ΔCC +
Gag: - + + + + + + -
IP Ab to:
IB Ab to:
p24
Myc-Ub
84
55
31
None
55
p24
31
25
None
55
Flag
None
84
HA

Fig. 7c

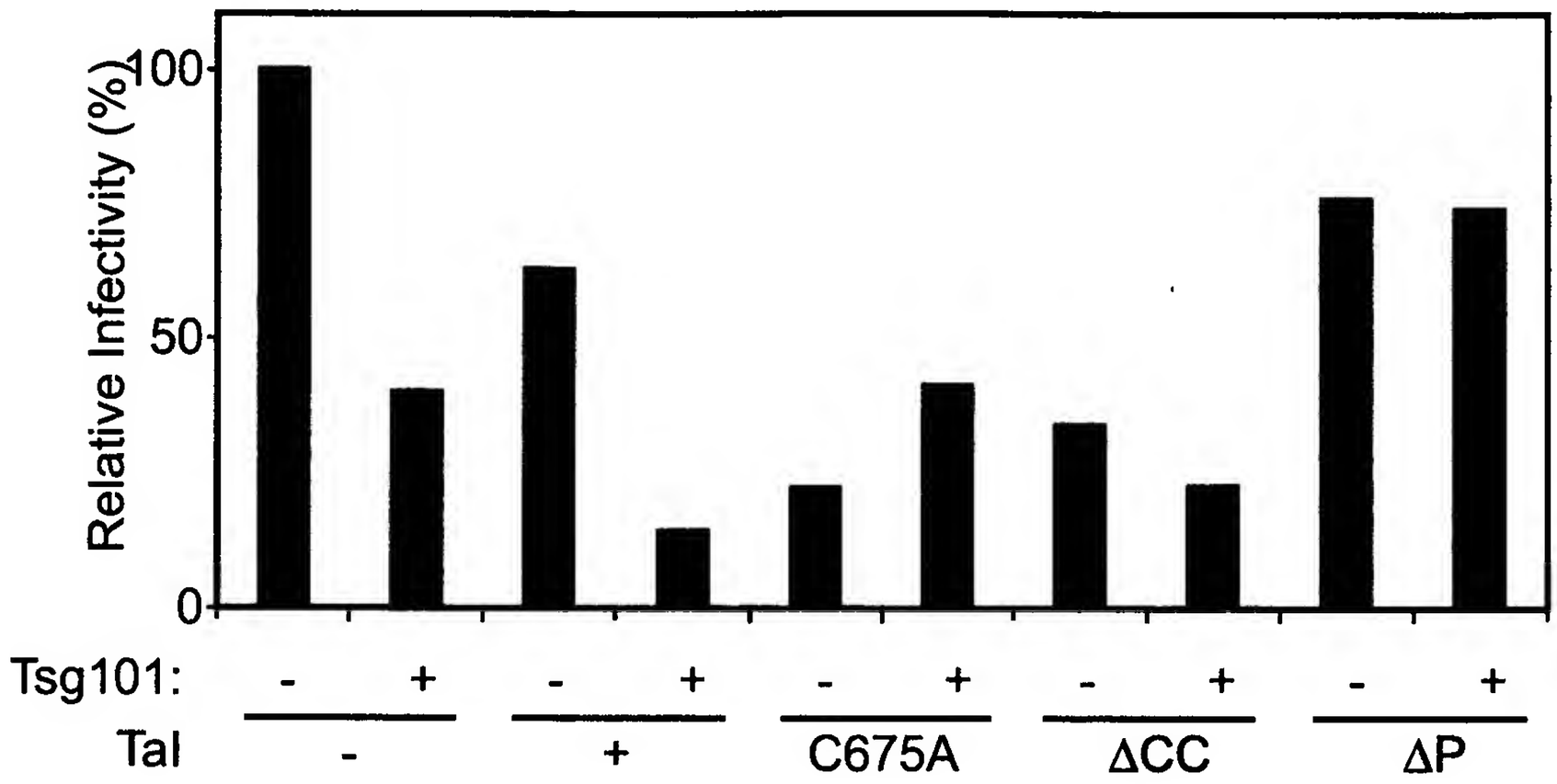

Relative Infectivity (%)
100
50
0
Tsg101: - + - + - + - + - +
Tal - + C675A ΔCC ΔP

Fig. 7d

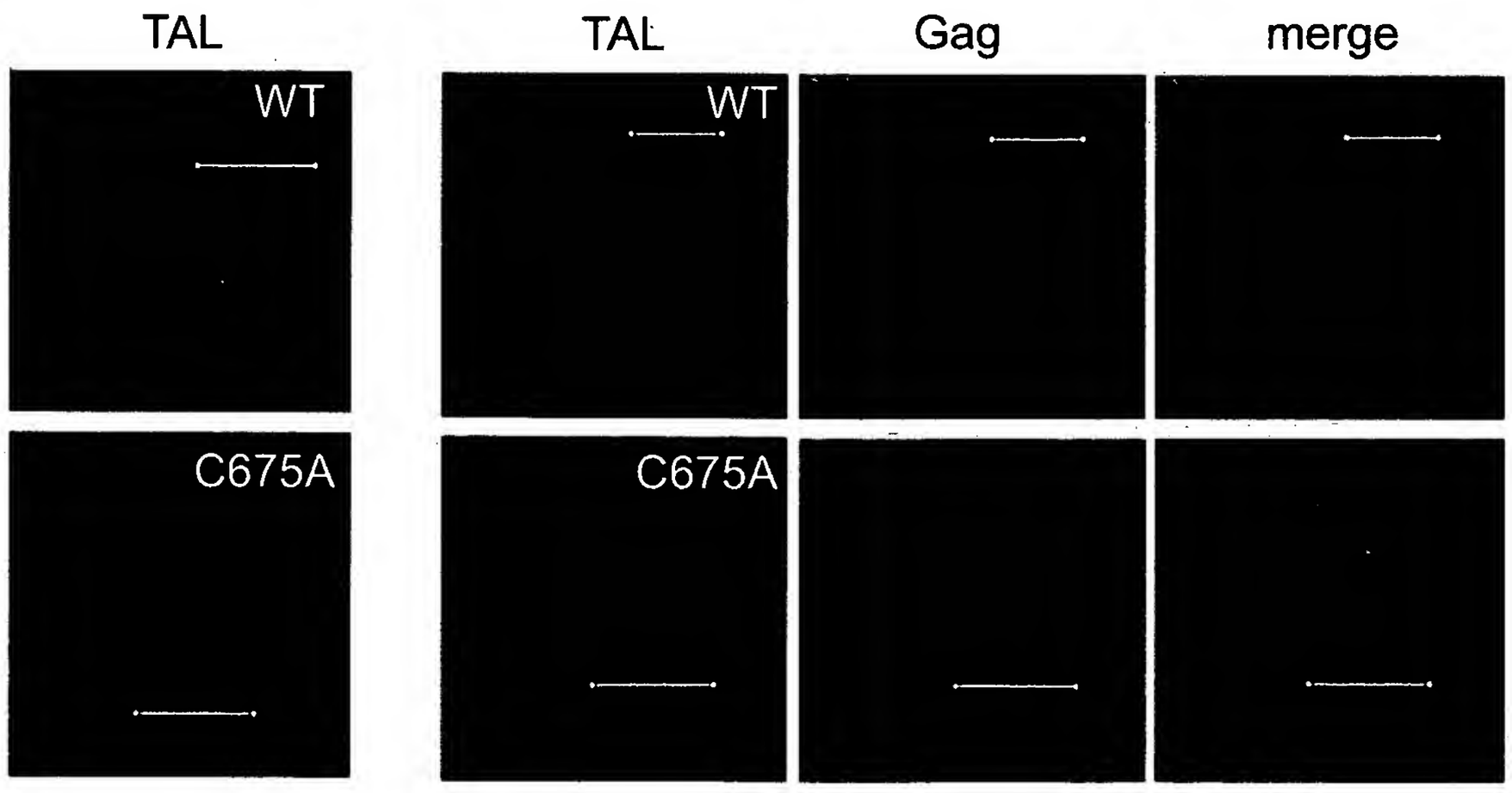

TAL
TAL
Gag
merge
WT
WT
C675A
C675A

Fig. 7e

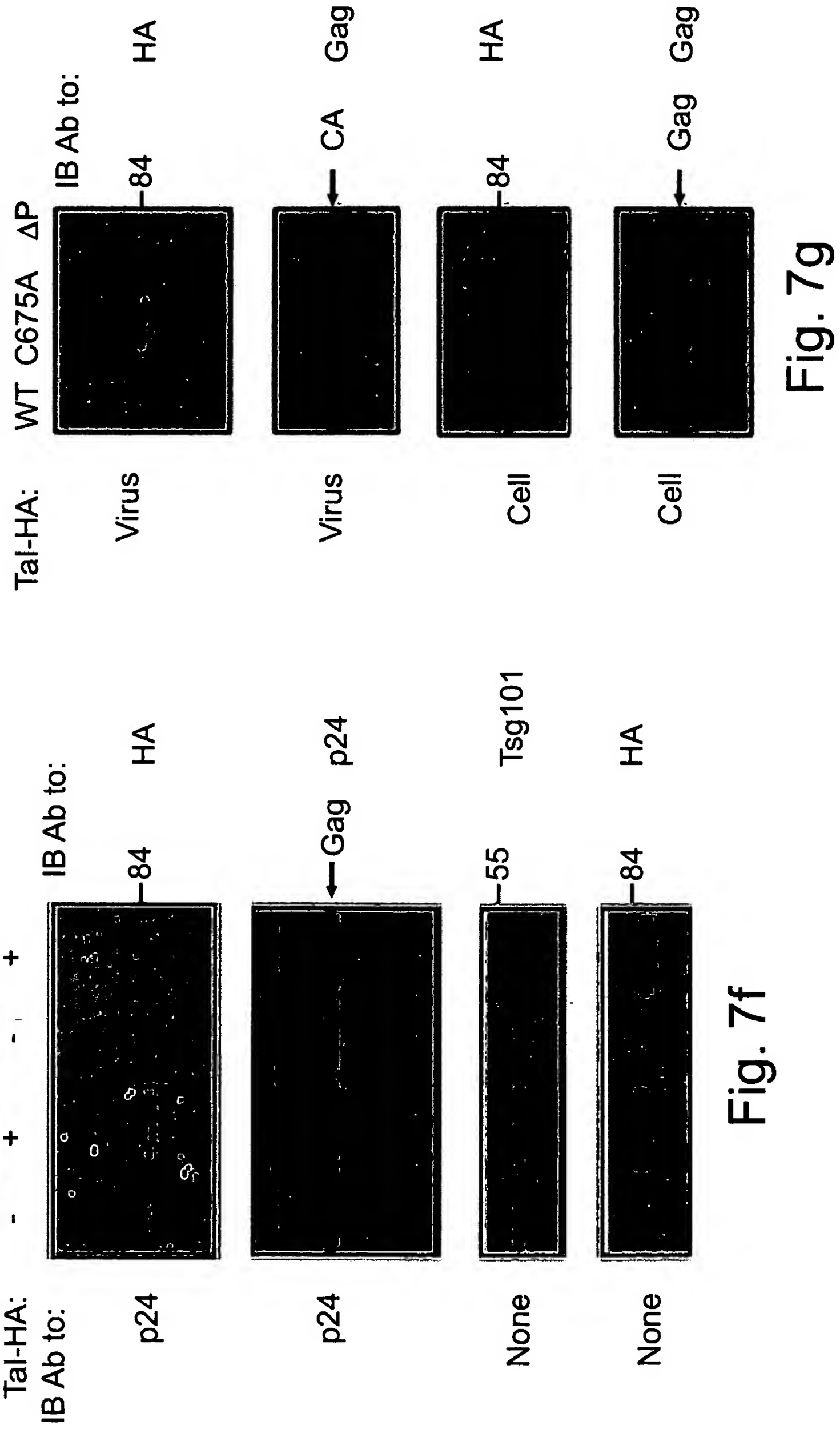

WT C67
Tal-HA:
Virus
Virus
Cell
Cell
IB Ab to:
HA
84
CA
Gag
HA
84
Gag
Gag
siRNa:
Tal-HA:
IB Ab to:
control
Tsg101
- + - +
p24
p24
None
None
Gag p24
Tsg101
55
84
HA

Fig. 7g

Fig. 7f

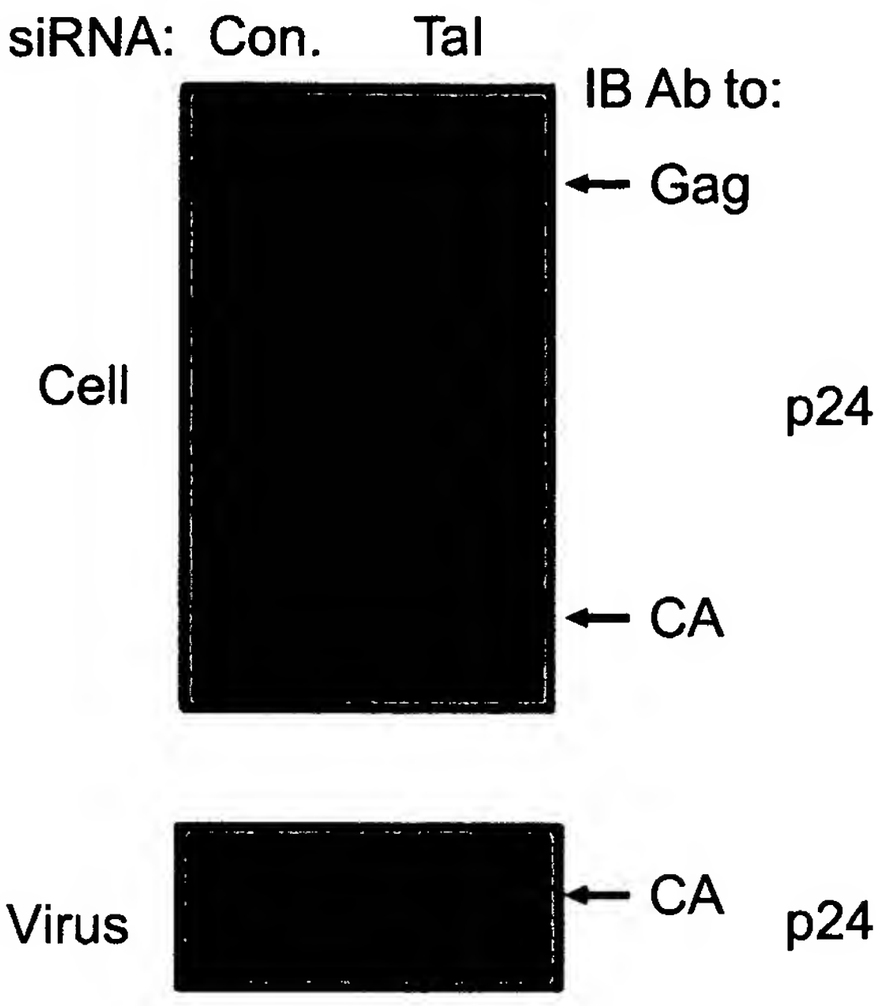

siRNA: Con. Tal
IB Ab to:
Gag
Cell
p24
CA
Virus
CA
p24

Fig. 7h

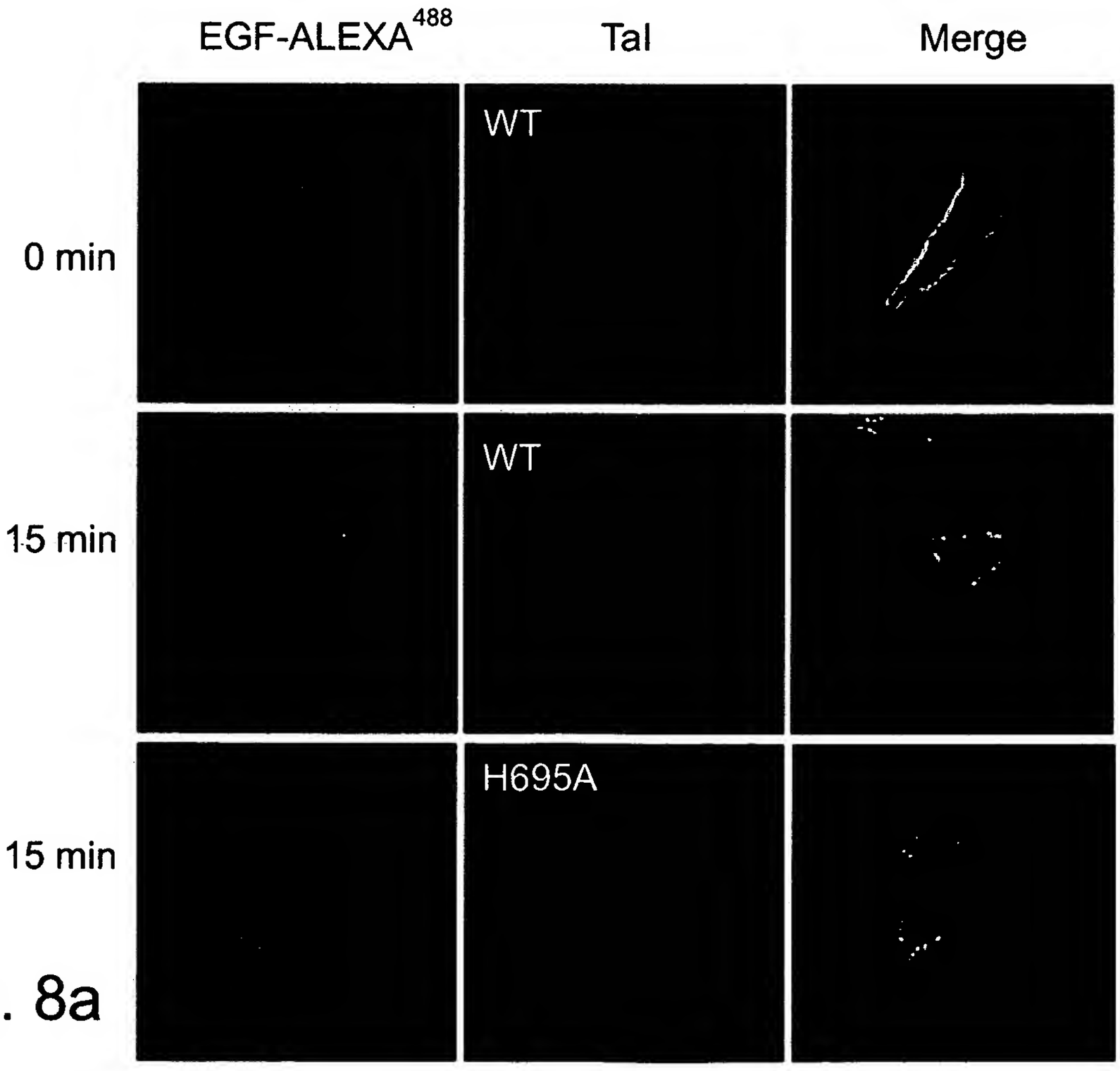

EGF-ALEXA488
Tal
Merge
0 min
WT
15 min
WT
15 min
H695A

Fig. 8a

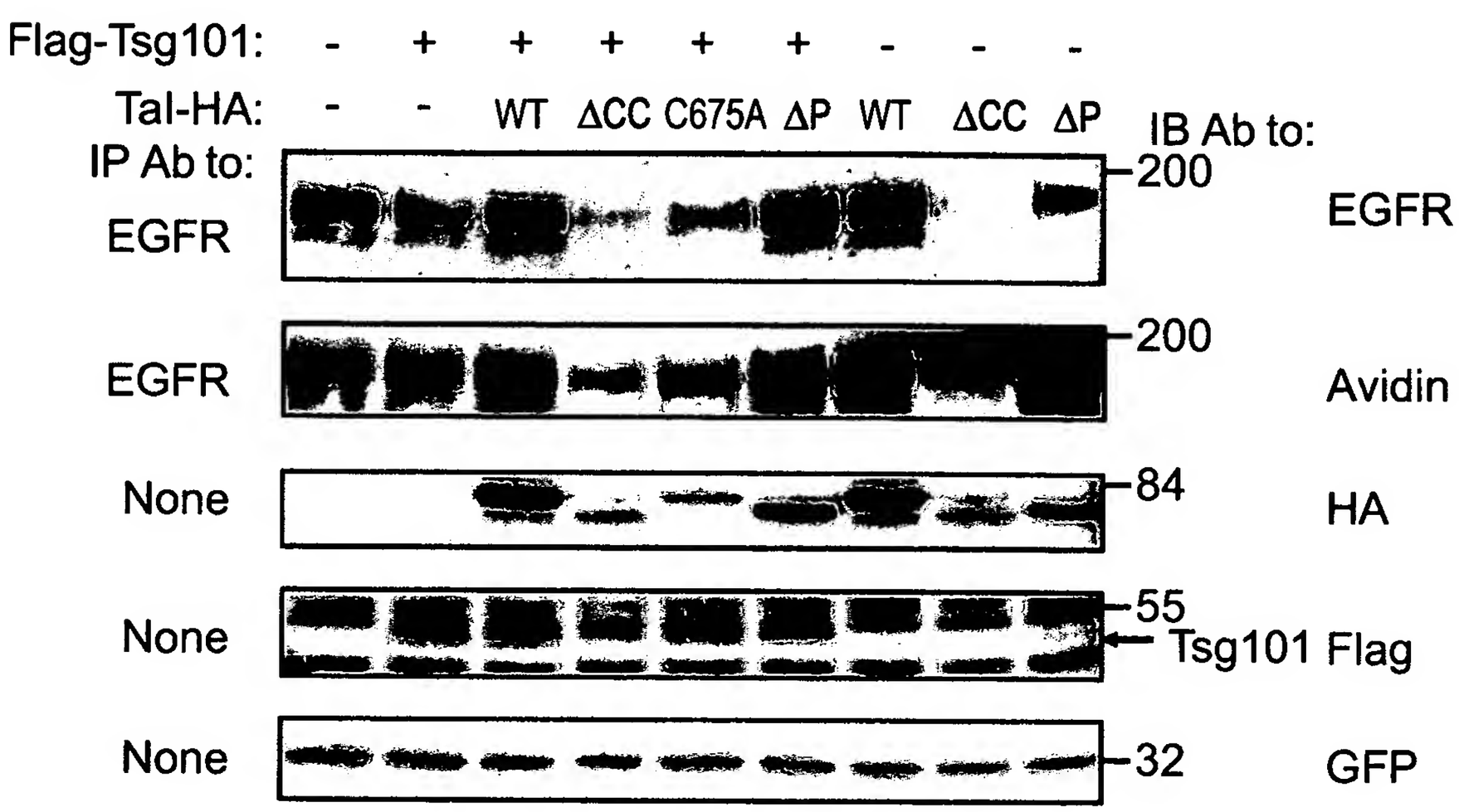

Flag-Tsg101: - + + + + + - - -
Tal-HA: - - WT ΔCC C675A ΔP WT ΔCC ΔP
IP Ab to:
EGFR
IB Ab to:
200
EGFR
EGFR
200
Avidin
None
84
HA
None
55
Tsg101 Flag
None
32
GFP

Fig. 8b

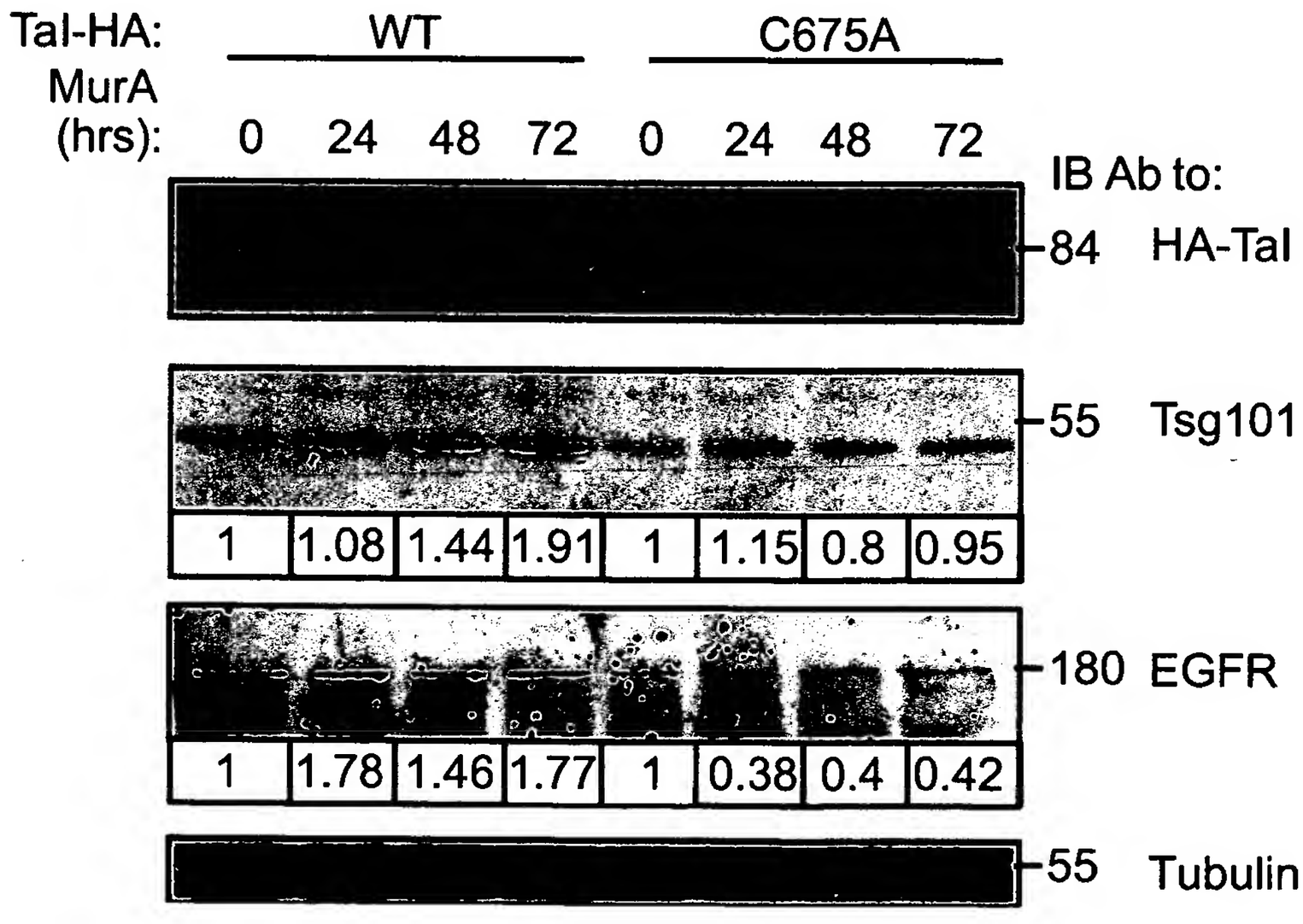

Tal-HA: WT C675A
MurA (hrs): 0 24 48 72 0 24 48 72
IB Ab to:
84 HA-Tal
55 Tsg101
1 1.08 1.44 1.91 1 1.15 0.8 0.95
180 EGFR
1 1.78 1.46 1.77 1 0.38 0.4 0.42
55 Tubulin

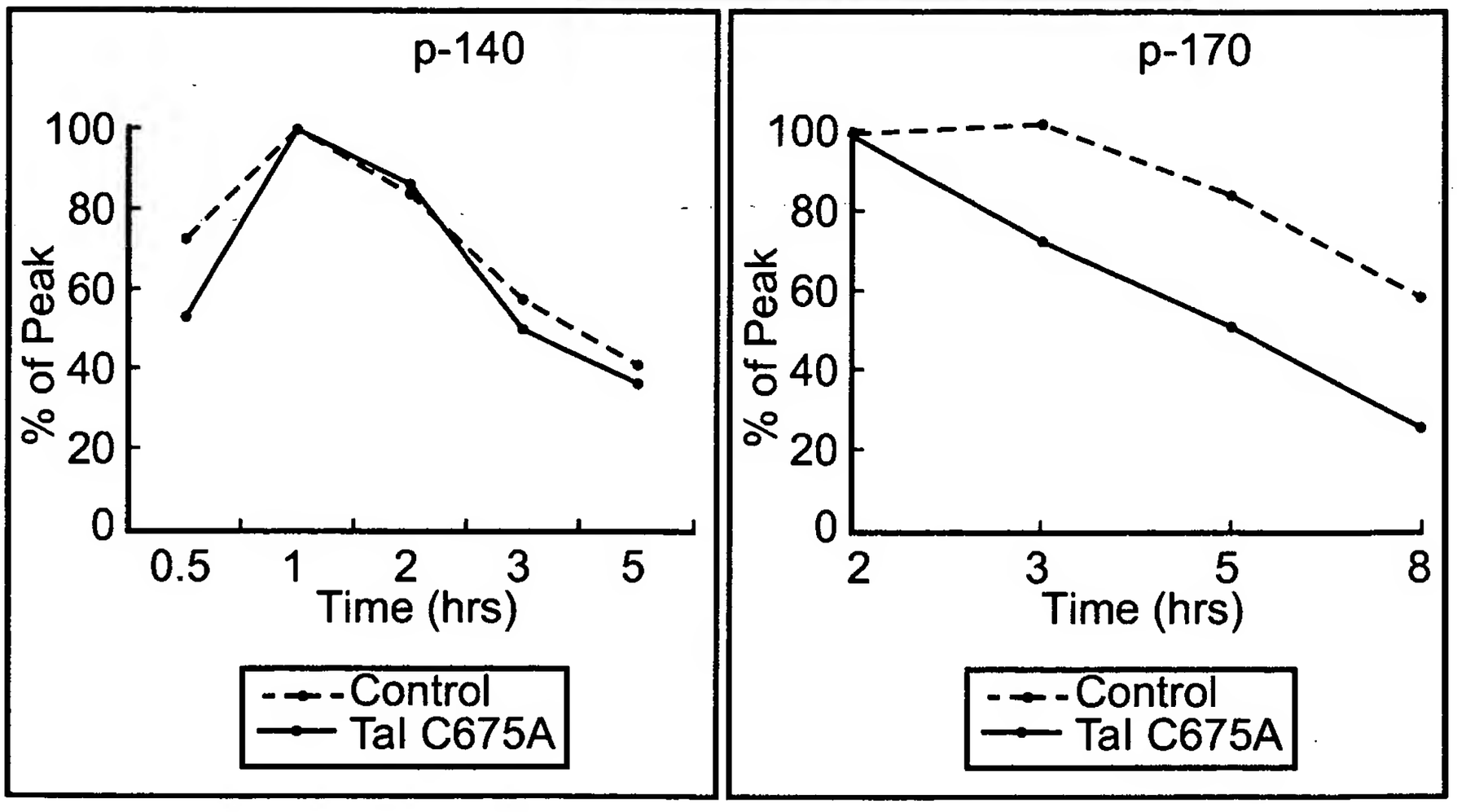
Fig. 8c


siRNA: control Tal-1252
EGF: - + - +
IB Ab to:
180
EGFR
84
Tal
55
Tubulin
siRNA: Tal-272 control
EGF: - + - +
IB Ab to:
180
EGFR
84
Tal
55
Tubulin

Fig. 8d


Time (hrs) 0.5 1 2 3 5 8
IP Ab to:
EGFR
Control
EGFR
C675A-Tal

Fig. 8e


p-140
100
80
60
40
20
0
% of Peak
0.5 1 2 3 5
Time (hrs)
Control
Tal C675A
p-170
100
80
60
40
20
0
% of Peak
2 3 5 8
Time (hrs)
Control
Tal C675A

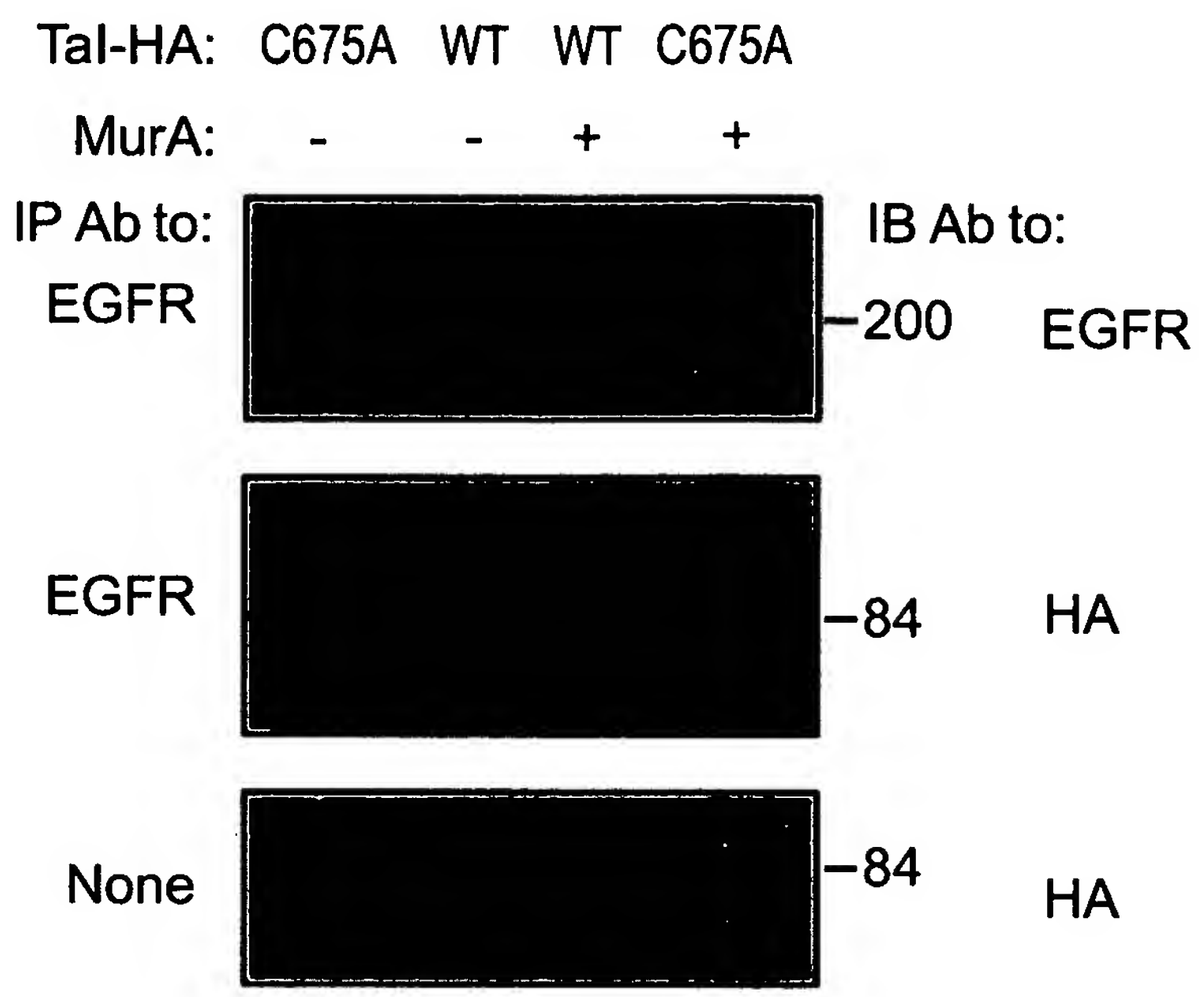

Tal-HA: C675A WT WT C675A
MurA: - - + +
IP Ab to:
EGFR
IB Ab to:
EGFR
200
EGFR
EGFR
84
HA
None
84
HA

Fig. 8f

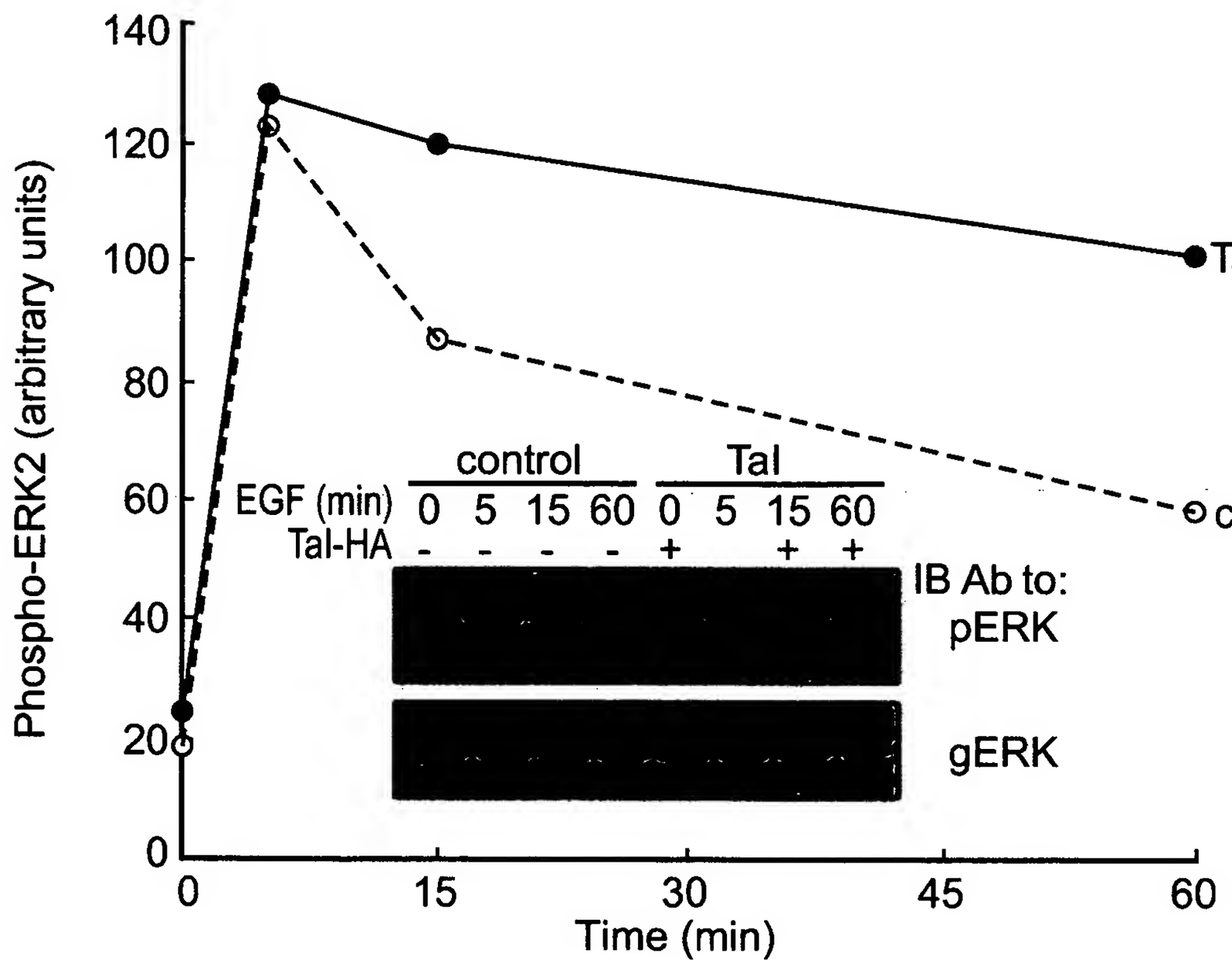

Phospho-ERK2 (arbitrary units)
140
120
100
80
60
40
20
0
0
15
30
45
60
Time (min)
Tal
control
control
Tal
EGF (min) 0 5 15 60 0 5 15 60
Tal-HA - - - - + + +
IB Ab to:
pERK
gERK

Fig. 8g

SEQ ID No.: 51

GFP—**EVVTPTAPQEPPESVRPSAPPAE**

Fig. 9a

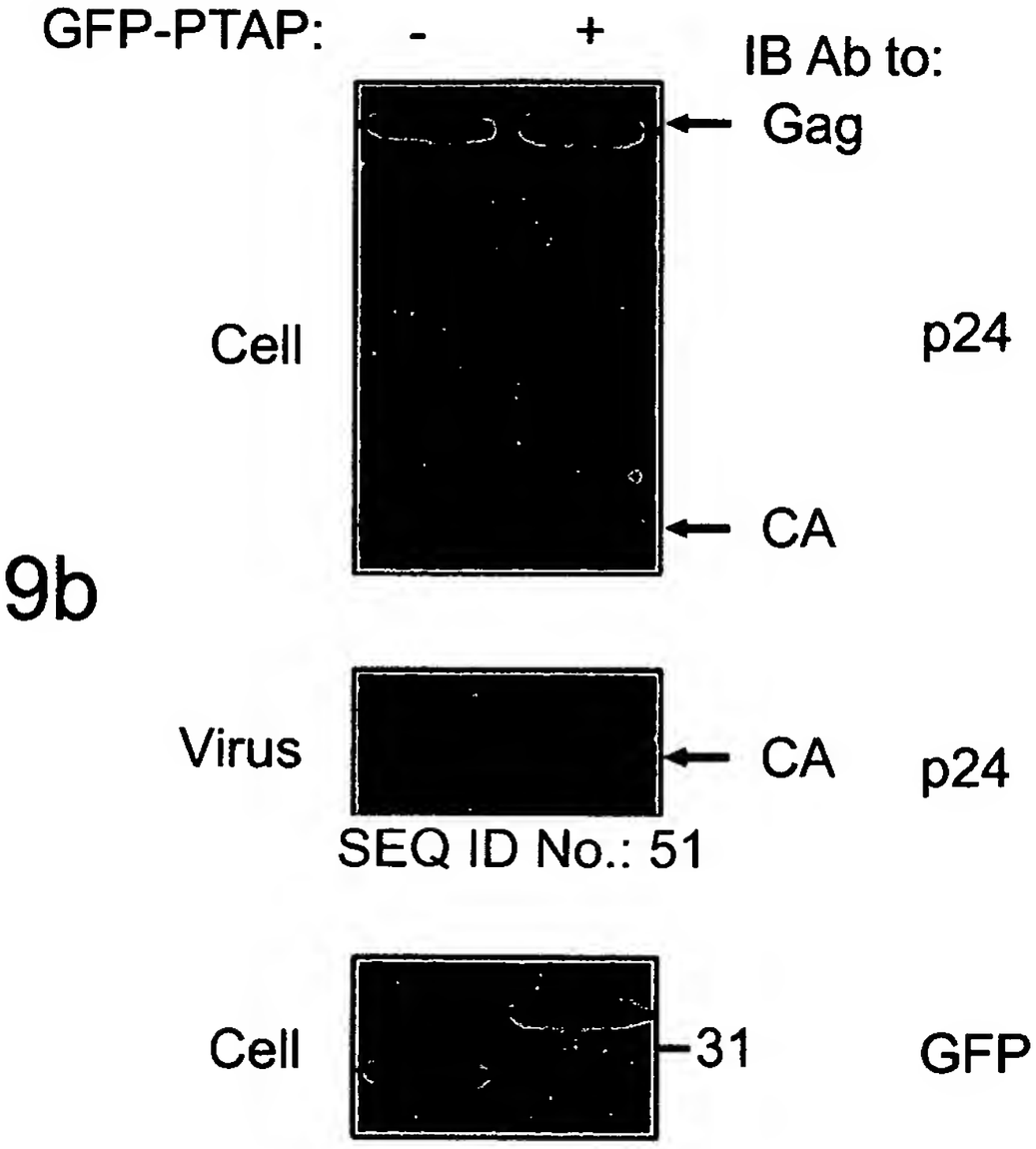


Fig. 9b

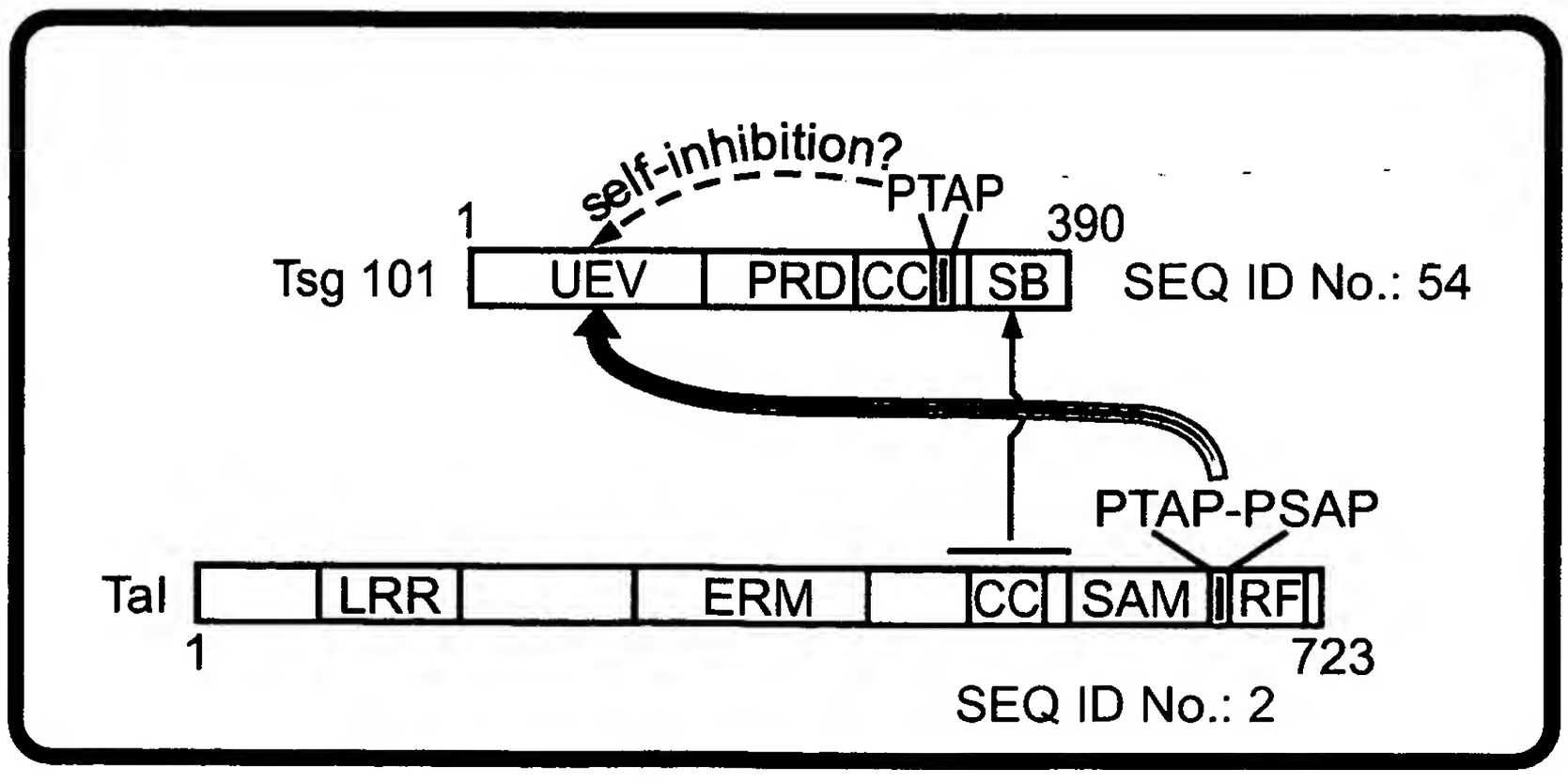


Fig. 10

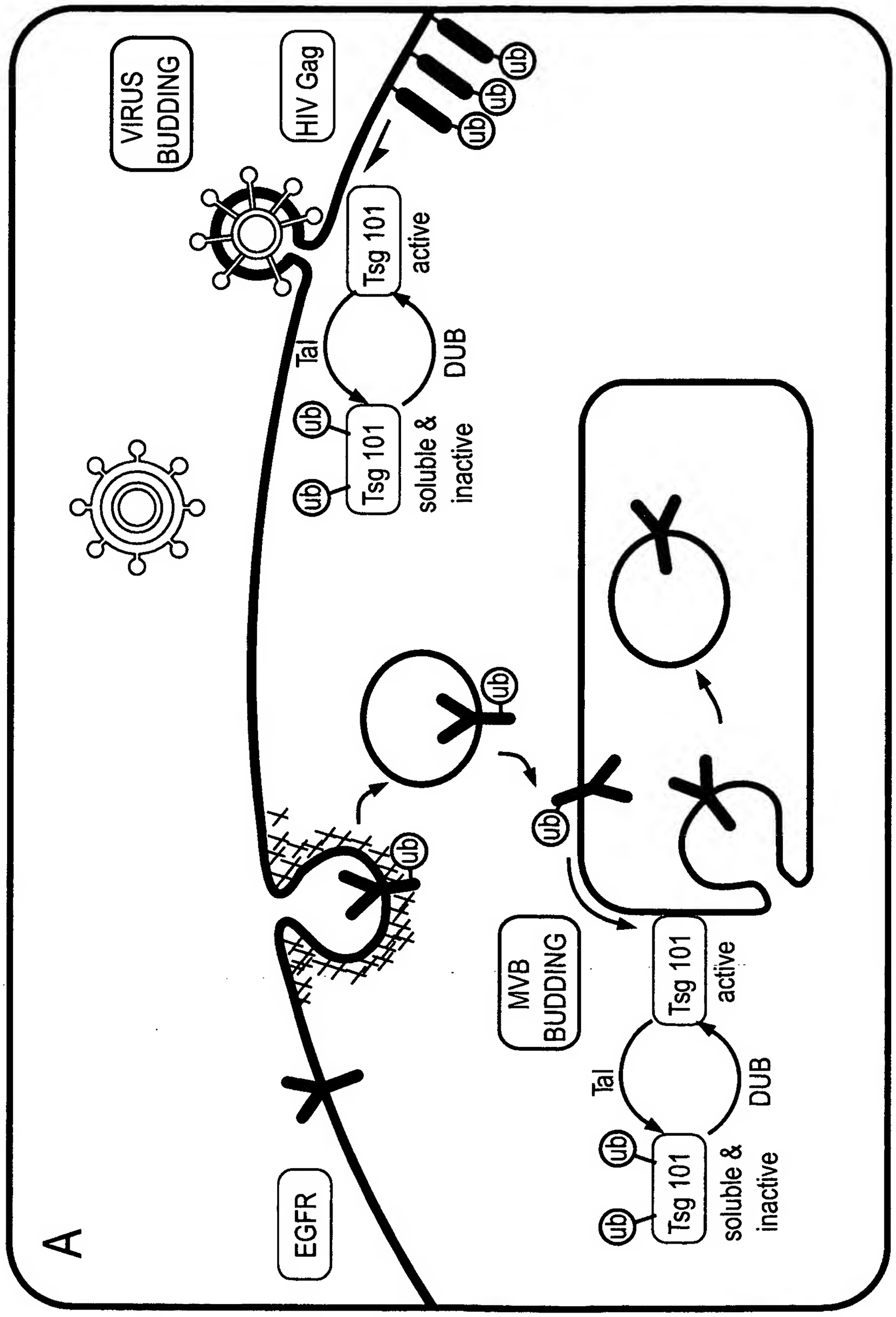

A
EGFR
VIRUS BUDDING
HIV Gag
Tsg 101
active
Tal
DUB
ub
soluble & inactive
MVB BUDDING

FIG. 11